THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR: Qingping Feng
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Location of experimental work to be carried out: Building(s) MSB Room(s) 253

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR. HSFO
GRANT TITLE(S): 1. Rac1 signaling in myocardial TNF-α expression in sepsis.
2. Heart development in diabetes: Role of NO
3. Cardioprotection by erythropoietin: Role of NO

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Sharon Lu
Lily Xiang
Yin Liu
Ting Zhang
Carmen Leung
Paul Arnold
Hoda Moazzen
Yan Wu
Zhi-Xin Sham
Murong Liu
Houxiang Hu

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☑ YES O NO
(including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ☒ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Adenovirus	☑ Yes O No	O Yes ☑ No	O Yes ☑ No	0.1 L	Applied Biomedical Materials	O 1 ☑ 2 O 3
Lentivirus	☑ Yes O No	O Yes ☑ No	O Yes ☑ No	0.1L	Applied Biomedical Materials	O 1 ☑ 2 O 3
(055:B5) E. coli	☑ Yes O No	☑ Yes O No	☑ Yes O No	0.2 L	ATCC	O 1 ☑ 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☑ YES O NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ☒ No		Not applicable
Rodent	☑ Yes O No	Mice heart, skin	2007-011-03
Non-human primate	O Yes ☑ No		
Other (specify)	O Yes ☑ No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☑ Yes ☒ No		
Rodent	☑ Yes O No		
Non-human primate	O Yes ☑ No		
Other (specify)	O Yes ☑ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ☑ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☑ YES ☑ NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below ☑ NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be made? ☑ YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Adenovirus	-	ABM	- Cre, nNOS	- gene knock down or upregulation
Lentivirus	- Lentivirus Expression System	ABM	- Oct 3/4, Tbx5, Gata4 Bmp60c, Nkx2.5, Nanog	

* Please attach a Material Safety Data Sheet or equivalent. ★ Adenovirus previously registered

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ☑ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ☑ NO
- SV 40 Large T antigen O YES ☑ NO
- E1A oncogene O YES ☑ NO
- Known oncogenes O YES, please specify _____ ☑ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☑ NO

4.5 Will virus be replication defective? ☑ YES O NO

4.6 Will virus be infectious to humans or animals? ☑ YES O NO

4.7 Will this be expected to increase the containment level required? O YES ☑ NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ☑ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ☑ YES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used C57 BL/6 mice

6.3 AUS protocol # 2007-011-03

6.4 Will any of the agents listed in section 4.0 be used in live animals ☑ YES, specify: Lentivirus O NO

6.5 Will the agent(s) be shed by the animal: ☑ YES O NO, please justify:

_____

_____

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7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES ☑ NO
- Pound source cats O YES ☑ NO
- Cattle, sheep or goats O YES ☑ NO
- Non-human primates O YES, please specify species _____ ☑ NO
- Wild caught animals O YES, please specify species & colony # _____ ☑ NO
- Birds O YES ☑ NO
- Others (wild or domestic) O YES, please specify _____ ☑ NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ☑ NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☑ NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

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10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ⊘ YES, please give country of origin_____
If no, please proceed to Section 12.0 ⊘ NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES ⊘ NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE Chris Lees

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13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 ☑ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus B10 - UWO - 0089
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, 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 Chris Jones Date: March 29, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

Contact UWO Health Services for medical evaluation.
_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

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Containment Level 2+ 3 Operational – Additional Items Checklist

Q.4. **(Chapter 3.1.3)**	Is a program in place (with appropriate authority to oversee safety and containment practices) for the management of biological safety issues?	MANDATORY ☑ Yes ☐ No ☐ N/A
Q.19. **(Chapter 3.1.3)**	Is a containment check performed before entering the containment laboratory (e.g. verify correct readings on the pressure monitoring device)?	MANDATORY ☑ Yes ☐ No ☐ N/A
Q.20. **(Chapter 3.1.3)**	Does personnel remove street clothing and change into dedicated laboratory clothing and shoes when entering the laboratory? Note: the use of full coverage protective clothing is an acceptable alternative. Laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to remove street clothing.	MANDATORY ☐ Yes ☑ No ☐ N/A
Q.24. **(Chapter 3.1.3)**	If an additional layer of protective clothing (e.g. solid-front gowns with tight-fitting wrists, gloves, respiratory protection) is worn over laboratory clothing when handling infectious materials, is it removed after completion of work (e.g. dedicated for use at the BSC)?	RECOMMENDED ☐ Yes ☑ No ☐ N/A
Q.25. **(Chapter 3.1.3)**	Are dedicated laboratory clothing and shoes removed before leaving the laboratory in a manner that minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing?	MANDATORY ☐ Yes ☑ No ☐ N/A
Q.34. **(Chapter 3.1.3)**	Are infectious agents stored inside the containment laboratory?	RECOMMENDED ☑ Yes ☐ No ☐ N/A
Q.35. **(Chapter 3.1.3)**	If agents are stored outside of the containment laboratory are they kept in leakproof containers in a restricted area? Note: emergency response procedures must take into account the existence of infectious agents that are stored outside of the containment area.	MANDATORY ☐ Yes ☐ No ☑ N/A

Containment Level 2+ 3 Operational – Additional Items Checklist

Q.36. **(Chapter 3.1.3)**	Are all activities with infectious materials conducted in a BSC?	**RECOMMENDED** ☑ Yes ☐ No ☐ N/A
Q.37. **(Chapter 3.1.3)**	Are other primary containment devices in combination with personal protective clothing and equipment used if it is not possible to conduct all activities with infectious materials in a BSC?	**MANDATORY** ☑ Yes ☐ No ☐ N/A
Q.38. **(Chapter 3.1.3)**	Is the centrifugation of infectious materials carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC?	**MANDATORY** ☐ Yes ☐ No ☑ N/A
Q.129. **(Chapter 3.1.3)**	In the event of an emergency, is an exit protocol in place whereby routine procedures might be bypassed? Note: in the event of life-threatening emergencies, personal health and safety are always a priority.	**MANDATORY** ☑ Yes ☐ No ☐ N/A
Q.130. **(Chapter 3.1.3)**	Has a reporting area been identified where further steps might be taken (e.g. disinfecting footwear, changing, showering)?	**MANDATORY** ☑ Yes ☐ No ☐ N/A
Q.138. **(Chapter 3.1.3)**	Are the general operational protocols supplemented with protocols specific to each project in progress?	**MANDATORY** ☑ Yes ☐ No ☐ N/A
Q.140. **(Chapter 3.1.3)**	Have personnel demonstrated proficiency in microbiological practices and techniques?	**MANDATORY** ☑ Yes ☐ No ☐ N/A

Western

Workplace Health

Policy and Procedure for persons working with Lentivirus and Lentivirus-based vectors

The potential for human inoculation and uncertainties concerning the potential infectivity of agents transduced by the Lentivirus system dictate the need for a simple medical surveillance program.

All persons working with Lentivirus and Lentivirus-based vectors will be seen at Workplace Health prior to the commencement of work with the virus.

A reference serum specimen will be collected and banked.

No annual follow up will be required.

In the event of a needle stick injury in which self-inoculation with Lentivirus or Lentivirus-based vectors is a possibility, the individual will report to Workplace Health and a serum sample for HIV and / or other pathogens will be obtained and tested. All serum specimens will be coded and the test result will be known only to the Workplace Health physician and the donor individual.

Approved Biohazards Committee December 6, 2007

Applied Biological Materials Inc.
MATERIAL SAFETY DATA SHEET - BIOLOGICAL SUBSTANCES

Date Updated: 09/03/2008
Version 1.0

SECTION I - BIOLOGICAL AGENT

NAME: Lentivirus Expression System

SOURCE NAME: *Human Immunodeficiency Virus*
SYNONYM OR CROSS REFERENCE: HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV

GENERAL CHARACTERISTICS: Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase

RECOMBINANT CHARACTERISTICS: 3rd-generation of self-inactivating recombinant lentiviral vectors with enhanced biosafety and minimal relation to the wild-type, human HIV-1 virus. The lentiviral particles produced with this system are replication-incompetent and designed with a number of safety features to enhance its biosafety.

An enhancer deletion in the U3 region of 3′ ΔLTR ensures self-inactivation of the lentiviral vector following transduction and integration into genomic DNA of the target cells. Utilization of an RSV promoter upstream of 5′LTR allows efficient Tat-independent production of viral RNA.

The number of lentiviral genes necessary for packaging, replication and transduction is limited to three (*gag, pol, rev*), and their expression is derived from different plasmids, which all lack packaging signals. These plasmids share no significant homology to the expression vector, preventing the generation of replication-competent virus. None of the *gag, pol,* or *rev* genes will be present in the packaged viral genome, thus making the mature virus replication-incompetent.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency

EPIDEMIOLOGY: First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

INCUBATlON PERIOD: Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years

COMMUNICABILITY: Period of communicability extends from asymptomatic period through appearance of opportunistic diseases

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Several reverse transscriptase and protease inhibitors now licensed

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PHYSICAL INACTIVATION: Effectiveness of 56·C - 60·C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56·C before serologic testing reduces residual infectivity to below detectable levels

SURVIVAL OUTSIDE HOST: Drying in environment causes rapid (within several hours) 90-99% reduction in HIV concentration

SECTION V - MEDICAL

SURVEILLANCE: Serological monitoring for evidence of HIV infection

FIRST AID/TREATMENT: Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV

IMMUNIZATION: None available

PROPHYLAXIS: Experimental prophylaxis with AZT/DDI or other appropriate drug

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 5 reported laboratory-acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwide

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, CSF, other specimens containing visible blood, unscreened or inadequately treated blood products

PRIMARY HAZARDS: Direct contact with skin and mucous membranes of the eye, nose and mouth; accidental parenteral inoculation; ingestion; hazard of aerosols exposure unknown

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and splashing infected materials - virus should be presumed in/on all equipment and devices coming in direct contact with infected materials
SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and non-cultured procedures (primary containment devices may be indicated eg. biological safety cabinets) and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV; Biosafety level 3 practices, containment equipment and facilities for all work culturing HIV

PROTECTIVE CLOTHING: Gloves should be worn when handling potentially infectious specimens, cultures or tissues; laboratory coats, gowns or suitable protective clothing should be worn

OTHER PRECAUTIONS: Keep hands away from the eyes, nose and mouth in order to avoid potential exposure of the mucous membranes; eye goggles or face shields may assist in accomplishing this objective

SECTION VIII – HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towels and apply 1% sodium hypochlorite, starting at perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate before disposal - steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labeled

SECTION IX – MISCELLANEOUS INFORMATION

The information contained in this Material Safety Datasheet is believed to be accurate but it is the responsibility of the user or supplier to determine the applicability of these data to the formulation of necessary safety precautions.

Applied Biological Materials Inc. shall not be held responsible for any damage resulting from the use of the above product or the information contained in this Material Safety Datasheet.

Biohazard Summary:

Adenovirus

How it is stored:

- Adenovirus' are stored in DMEM media with 2.5% glycerol at -80°C.

How it is used:

- Amplification is performed using 293 cells by infecting the cells with 10µl of the adenovirus in a 60mm dish. The collection of cells are frozen and thawed three times, and the supernatant is separated from cell debris for infection of target cells.

How it is disposed:

- Any solution or containers that handled adenovirus are washed with bleach and autoclaved before disposal.

Lentivirus

How it is stored:

- Lentivirus' are stored in 10mM Tris-HCl, 1mM EDTA, pH8.0 buffer at -80°C.

How it is used:

- Cocktails containing the 6 lentivirus' are transfected into neonatal mice fibroblasts or adult mice skin cells. Cells are monitored for viability and characteristic changes.
- Upon approval of the protocol from the UWO animal use subcommittee, we will inject transfected cells that demonstrate characteristic changes into live animals for heart failure therapy.

How it is disposed:

- Any solution or containers that handled lentivirus are washed with bleach and autoclaved before disposal.

Escherichia coli

How it is stored:

- E. coli is stored in glycerol medium at -80°C.

How it is used:

- E. coli is amplified in nutrient agar or broth at 37°C for approximately 18hrs.

- E. coli is injected intravenously or intraperitoneally into mice at a dose of $2.5x10^7$ CFU/g of body weight to induce gram-negative sepsis conditions. Mice are then monitored and analyzed as per experiment protocol.

How it is disposed:

- Any container that handled E. coli solution is washed with bleach and autoclaved before disposal.
- All animals treated with E. coli are incinerated after sacrifice or death.

Viral Vector Information:

pLenti-III-m-Gata4

Accession Number	NM_008092
Vector	pLenti-III-HA
Backbone Size	8183bp
Description	This gene encodes a member of the GATA family of zinc-finger transcription factors. Members of this family recognize the GATA motif which is present in the promoters of many genes. This protein is thought to regulate genes involved in embryogenesis and in myocardial differentiation and function. Mutations in this gene have been associated with cardiac septal defects.
Species of Genes	Mouse
Host Strain	DH5alpha
Cloning Sites	EcoRI
Insert Size	1.9kb
Promoter	CMV
Tags	HA
Selection Marker	Kanamycin
Sequence Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid
Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
StorageConditions	1 year when stored at -20°C or lower in a non-frost free freezer

pLenti-III-m-Nanog

Accession Number	NM_008092
Vector	pLenti-III-HA
Backbone Size	8183bp

Description	This gene encodes a member of the GATA family of zinc-finger transcription factors. Members of this family recognize the GATA motif which is present in the promoters of many genes. This protein is thought to regulate genes involved in embryogenesis and in myocardial differentiation and function. Mutations in this gene have been associated with cardiac septal defects.
Species of Genes	Mouse
Host Strain	DH5alpha
Cloning Sites	EcoRI
Insert Size	1.9kb
Promoter	CMV
Tags	HA
Selection Marker	Kanamycin
Sequence Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid
Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
StorageConditions	1 year when stored at -20°C or lower in a non-frost free freezer

pLenti-III-m-NKX2.5

Accession Number	BC139303
Vector Backbone Name	Please inquire
Vector Backbone Size (bp)	8119bp
Species of Gene(s)	Mouse
Host Strain	DH5a
Cloning Sites	BamHI/XhoI
Insert Size (bp)	2kb
Promoter	CMV
Fusion Proteins or Tags	HA

Selection Marker	Kan
Sequencing Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid
Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
Storage Conditions	1 year when stored at -20°C or lower in a non-frost free freezer

pLenti-III-m-Oct 3/4

Accession Number	NM_013633
Vector Backbone Name	Please inquire
Vector Backbone Size (bp)	8183bp
Species of Gene(s)	Mouse
Host Strain	DH5a
Cloning Sites	EcoRI
Insert Size (bp)	1324bp
Promoter	CMV
Fusion Proteins or Tags	HA
Selection Marker	Kan
Sequencing Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid
Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
Storage Conditions	1 year when stored at -20°C or lower in a non-frost free freezer

pLenti-III-m-Tbx5

Accession Number	BC090639
Vector Backbone Name	Please inquire

Vector Backbone Size (bp)	8183bp
Species of Gene(s)	Mouse
Host Strain	DH5a
Cloning Sites	NotI/SalI
Insert Size (bp)	3.2kb
Promoter	CMV
Fusion Proteins or Tags	HA
Selection Marker	Kan
Sequencing Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid
Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
Storage Conditions	1 year when stored at -20°C or lower in a non-frost free freezer

pLenti-III-m-Baf60c

Accession Number	BC060525
Vector Backbone Name	Please inquire
Vector Backbone Size (bp)	8183bp
Species of Gene(s)	Mouse
Host Strain	DH5a
Cloning Sites	SalI/NotI
Insert Size (bp)	1.6kb
Promoter	CMV
Fusion Proteins or Tags	HA
Selection Marker	Kan
Sequencing Primer	CGCAAATGGGCGGATGGCGTG
Format	Plasmid

Appearance	Clear Liquid
Storage Buffer	10mM Tris-HCI, 1mM EDTA, pH8.0
Storage Conditions	1 year when stored at -20°C or lower in a non-frost free freezer