

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: July 9, 2010  
Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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, 1<sup>st</sup> 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 Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto: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/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR	<u>Qingping Feng</u>
DEPARTMENT	<u>PHYSIOLOGY AND PHARMACOLOGY</u>
ADDRESS	<u>MSB 254</u>
PHONE NUMBER	<u>82989</u>
EMERGENCY PHONE NUMBER(S)	<u>519-933-9289</u>
EMAIL	<u>Qingping.feng@schulich.uwo.ca</u>

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 15.0, Approvals).

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

List all personnel working under Principal Investigators supervision in this location:

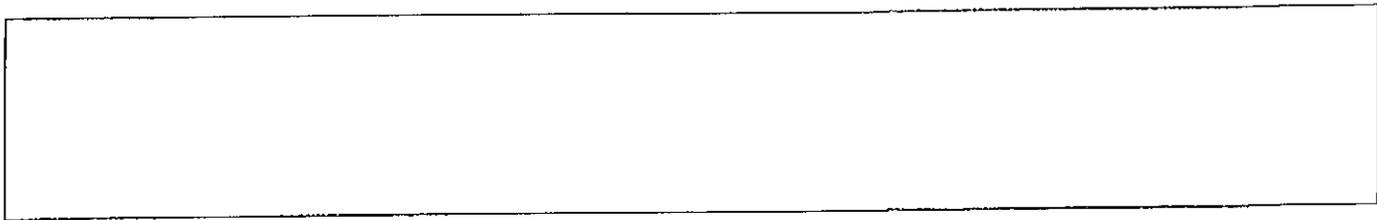
<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Sharon Lu	<u>Sharon.lu@schulich.uwo.ca</u>	<u>July 14, 2008</u>
Lily Xiang	<u>Fxiang2@uwo.ca</u>	<u>May 26, 2008</u>
Yin Liu	<u>Yliu258@uwo.ca</u>	<u>Registered for refresher training</u>
Ting Zhang	<u>Tzhang53@uwo.ca</u>	<u>May 26, 2008</u>
Carmen Leung	<u>Cleung73@uwo.ca</u>	<u>Registered for refresher training</u>
Hoda Moazzen	<u>hmoazzen@uwo.ca</u>	<u>Registered for refresher training</u>
Yan Wu	<u>Ywu287@uwo.ca</u>	<u>Registered for refresher training</u>
Murong Liu	<u>Mliu223@uwo.ca</u>	<u>July 14, 2008</u>

**Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.**

Adenovirus' are stored in DMEM media with 2.5% glycerol at -80°C. Amplification of adenovirus is performed using HEK 293 cells by infecting the cells with 10 µl of the adenovirus in a 60 mm dish. The collection of cells are frozen and thawed three times and the supernatant is separated from cell debris for infection of target cells, which are neonatal cardiomyocytes. Any solution or containers that handled adenovirus are washed with bleach and autoclaved before disposal.

Lentivirus will be produced by the company Applied Biological Materials in Richmond, BC. The lentivirus will be purified and concentrated through ultracentrifugation by the company to produce a high titer. Lentivirus' are stored in 10 mM Tris-HCl, 1 mM EDTA, pH 8.0 buffer at -80°C. Cocktails containing a combination of 6 lentivirus', packaged in VSVG envelope, (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4, Nanog) are infected into isolated fibroblasts from one of four cell sources: mouse neonatal skin, mouse embryo E14.5, mouse neonatal heart and the mouse adult heart. The isolated fibroblasts are grown until confluent and transfected at passage 2 or 3. Cells are incubated with the lentivirus for 24 hours at 37°C and 5% CO<sub>2</sub> and each virus is used at a range of multiplicity of infections (MOIs) or 5-20. After 24 hours the lentivirus is removed from the cells and inactivated with 10% bleach. Cells are then monitored for viability and characteristic changes of differentiation from fibroblasts into cardiomyocytes. Following our *in vitro* studies with the lentivirus, we will establish which combination of lentivirus (Tbx5, Gata4, Baf60C, Nkx2.5, Oct3/4 and/or Nanog) will be most effective in reprogramming fibroblasts into cardiomyocytes. Upon approval of the protocol from the UWO animal subcommittee, we will inject infected cells that demonstrate characteristic changes into live animals for heart failure therapy. Specifically, the protocol will follow the details used in our *in vitro* work to develop viable cells with characteristic changes. The cells will then be injected into the pericardium cavity of mice and their ability to differentiate into cardiomyocytes will be evaluated using immunohistostaining. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. After the successful injection of infected cells into the heart of mice has been shown, the established combination of lentivirus from our *in vitro* studies will be directly injected into the pericardium cavity of mice to demonstrate reprogramming of cells into cardiomyocytes. Immunohistostaining will be used to evaluate the ability of cells to differentiate into cardiomyocytes. The mice will be sacrificed and their hearts will be fixed in paraformaldehyde and imbedded in paraffin. The hearts will then be sectioned onto slides and stained for cardiomyocyte-specific markers like α-actinin or troponin-I. Injection of lentiviral infected cells or direct injection of lentivirus into the cardiac region of mice will demonstrate *in vivo* reprogramming of cells into cardiomyocytes, which will be beneficial for heart function following myocardial infarction. Any solution or containers that handled lentivirus are washed with bleach and autoclaved before disposal.

The E.Coli bacteria will be stored at -80 degrees Celsius.  $2.5 \times 10^7$  clones/ g of E. coli bacteria will be injected into the penis vein of mice to induce sepsis and their survival will be monitored. At 3 days after bacterial injection the mice will undergo hemodynamic analysis and sacrificed and incinerated after. The E. coli bacteria will be inactivated with 10% bleach. Any solution or containers that handled the E. coli bacteria are washed with 10% bleach and autoclaved before disposal.



## Directed cellular reprogramming of cardiac fibroblasts to cardiomyocytes

Qingping Feng

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London, Ontario, Canada

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Tel. 519-850-2989

With cardiovascular disease as the global leading cause of death, the development of cellular therapies to regenerate a damaged heart is imperative. Following myocardial infarction (MI), large numbers of cardiomyocytes are lost and fibroblasts proliferate rapidly, leading to impaired heart function. Many autologous cell types have been studied for their innate cardiogenic potential but none have been truly successful. It has been demonstrated that cardiomyocytes can be derived from human embryonic stem cells and induced pluripotent stem cells. However, these cell types can form teratomas when transplanted due to their original pluripotent nature. Studies on transdifferentiation or cellular reprogramming using defined factors have been promising because an abundant cell type can be directly reprogrammed into another important cell type without first becoming pluripotent. The present study proposes that cardiac fibroblasts, which proliferate extensively after MI, could be directed to transdifferentiate into cardiomyocytes with cardiac specific genes, therefore potentially improving cardiac function. The objectives of this study are to evaluate the ability of cardiac fibroblasts to reprogram into cardiomyocytes and to investigate if transplantation of these reprogrammed fibroblasts can improve heart function post-MI in a mouse model. Different combinations of lentiviral transductions with *Baf60c*, *Gata4*, *Tbx5*, *Nkx2.5*, *Oct3/4* and *Nanog* genes will be performed on cultured fibroblasts and monitored for spontaneous beating. Subsequently, immunofluorescence and western blot analysis will be carried out to analyze cardiomyocyte specific protein expression. The reprogrammed cells will be transplanted into the peri-infarct region of the heart after MI and heart function will be measured by Millar pressure-volume relationships. It is expected that transplantation of the lentiviral transduced fibroblasts will lead to cardiomyocyte regeneration, improve cardiac repair and cardiac function post-MI. This study may have a major impact on the regenerative medicine and treatment of MI.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents?  YES  NO  
 (non-pathogenic and pathogenic biological agents 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?  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:

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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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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	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1L	Applied Biomedical Materials	<input type="radio"/> 1 <input type="radio"/> 2 <input checked="" type="radio"/> 2+ <input type="radio"/> 3
Lentivirus	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1L	Applied Biomedical Materials	<input type="radio"/> 1 <input type="radio"/> 2 <input checked="" type="radio"/> 2+ <input type="radio"/> 3
E. coli (055iB5)	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	0.2L	ATCC	<input type="radio"/> 1 <input type="radio"/> 2 <input checked="" type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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  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	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Mice heart, skin	2007-011-03
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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	<input checked="" type="radio"/> Yes <input type="radio"/> No	HEK 293	ATCC (already have)
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

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

### 3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials?  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		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 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?  YES, complete table below  NO

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

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

\*\* Please attach a plasmid map.

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Adenovirus	Adenoviral expression system	ABM	<b>Cre</b> (not oncogenic gene) Catalogue #: 000198A	Gene knockdown of specifically floxed genes; cells remain healthy and do not turn cancerous.
Lentivirus	Lentivirus expression system	ABM	<b>Oct 3/4</b> (Cat. #: LV010061), <b>Tbx5</b> (Cat. #: LV010060), <b>Gata4</b> (Cat. #: LV010058), <b>Baf60c</b> (LV010062), <b>Nkx2.5</b> (Cat. #: LV010059), <b>Nanog</b> (Cat. #: LV010063) (All from ABM and not oncogenic genes)	Gene upregulation; cells change phenotype to cardiomyocyte-like; cells remain health and do not turn cancerous.

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective?  YES  NO

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

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

## 5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent?  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?  YES  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?  YES, number: \_\_\_\_\_  NO  PENDING

## 6.0 Animal Experiments

Containment Level?

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

6.2 Name of animal species to be used C57BL/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  NO  
We are going to inject mice with lentivirus and lentivirus infected cells into mice.

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

### 7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including be used (see list below)?  YES  No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

### 8.0 Biological Toxins

8.1 Will toxins of biological origin be used?  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<sub>50</sub> (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?  YES, Please provide details: \_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

### 9.0 Insects

9.1 Do you use insects?  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?  Initiate and maintain colony, give location: \_\_\_\_\_  
 "One-time" use, give location: \_\_\_\_\_

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

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9.7 Do you use insects that require a permit from the CFIA permit?  YES  NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 10.0 Plants

10.1 Do you use plants?  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?  Grow and maintain a crop  "One-time" use

10.6 Do you do any modifications to the plant?  YES  NO

If yes, please describe: \_\_\_\_\_

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

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10.8 Is the CFIA permit attached?  YES  NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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### 11.0 Import Requirements

11.1 Will any of the above agents be imported?  YES, please give country of origin USA  NO

If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS?  YES, please provide permit # \_\_\_\_\_  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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE                     *Ort Jensen*

**13.0 Containment Levels**

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

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, permit # if on-campus BIO-UWO-0089  
 NO, please certify  
 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 Lewis* Date: Jan 31, 2011

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 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 biological agents listed, such as a needlestick injury:

The procedure according to Section 3.5 Medical Procedures and Incident Reporting of the UWO Biosafety Guidelines and Procedures Manual for Containment Level 1 and 2 Laboratories will be followed.

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

2) Safety Officer for the University of Western Ontario  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

3) Safety Officer for Institution where experiments will take place (if not UWO):  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:



Public Health  
Agency of Canada

Agence de la santé  
publique du Canada

**Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3<sup>rd</sup> Ed., 2004***

This letter serves to confirm that the Public Health Agency of Canada, Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

**HPTA Registration Number:** R-06-000598

**Facility:** University of Western Ontario  
Department of Physiology and Pharmacology

**Attention:** Dr. Qingping Feng

**Address:** 1151 Richmond Street  
London, ON  
N6A 3K7  
Tel: 519-661-2111 ext 82989

**Laboratory Room Number(s):** DSB 6027

**Type of work:**  *in vitro* only  
 *in vitro & in vivo* (small lab animals only)\*

**Compliance Letter Number:** C-00195

**Compliance Letter Expiry Date:** July 7, 2012.

To renew your compliance letter please complete a CL2 checklist and fax it to (613) 941-0596. The checklist can be obtained from the following website:  
[www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html](http://www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html)

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

Marianne Heisz  
Chief, Importation and Regulatory Affairs

JULY 20, 2010

Date

\*In vivo work is only authorized for mice, rats, guinea pigs and insects. The Pathogen Regulation Directorate must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses. Please contact CFIA for all in vivo work.

Public Health  
Agency of CanadaAgence de la santé  
publique du Canada**Canadian end-user compliance with the *Laboratory Biosafety Guidelines, 3<sup>rd</sup> Ed., 2004***

This letter serves to confirm that the Public Health Agency of Canada, Pathogen Regulation Directorate has reviewed a Containment Level 2 checklist for the facility identified below, and found the information submitted acceptable.

**HPTA Registration Number:** R-06-000598

**Facility:** University of Western Ontario  
Schulich School of Medicine & Dentistry  
Department of Physiology and Pharmacology

**Attention:** Dr. Qingping Feng

**Address:** Medical Science Building  
1151 Richmond Street  
London, ON  
N6A 5C1  
Tel: 519-661-2111 ext 82989

**Laboratory Room Number(s):** 253A

**Type of work:**  *in vitro* only  
 *in vitro* & *in vivo* (small lab animals only)

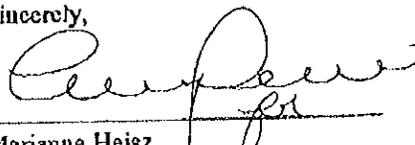
**Compliance Letter Number:** C-00047

**Compliance Letter Expiry Date:** April 27, 2012.

To renew your compliance letter please complete a CL2 checklist and fax it to (613) 941-0596. The checklist can be obtained from the following website:  
[www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html](http://www.phac-aspc.gc.ca/ols-bsl/pathogen/index.html)

Should you have any questions regarding this letter, please do not hesitate to contact our office at (613) 957-1779.

Sincerely,

  
Marianne Heisz  
Chief, Importation and Regulatory Affairs

MAY 25, 2010  
Date

\*In vivo work is only authorized for mice, rats, guinea pigs and insects. The Pathogen Regulation Directorate must be contacted prior to initiating any work involving domestic animals including poultry, cattle, sheep, swine and horses. Please contact CFIA for all in vivo work.

Canada

## Bacteria

ATCC <sup>®</sup> Number:	12014 <sup>™</sup> <input type="button" value="Order this Item"/>	Price:	\$155.00
Organism:	<i>Escherichia coli</i> (Migula) Castellani and Chalmers	<a href="#">Related Links ▶</a>	
Designations:	CDC 5624-50 [NCTC 9701]	<a href="#">NCBI Entrez Search</a>	
Depositor:	CDC	<a href="#">Make a Deposit</a>	
<b>Biosafety Level:</b>	2	<a href="#">Frequently Asked Questions</a>	
Shipped:	freeze-dried	<a href="#">Material Transfer Agreement</a>	
Growth Conditions:	<u>ATCC medium 3</u> : Nutrient agar or nutrient broth <b>Temperature</b> : 37.0°C	<a href="#">Technical Support</a>	
Permits/Forms:	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.	<a href="#">Related Products</a>	
Antigenic Properties:	serotype O55:K59(B5):H-		

[Return to Top](#)

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: 3<sup>rd</sup>-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

**INCUBATION 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 transcriptase 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.



## 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

----- Original Message -----

**Subject:**Re: Biological Agents Registry form (Feng lab)

**Date:**Mon, 07 Feb 2011 10:33:38 -0500

**From:**Carmen Leung <cleung73@uwo.ca>

**To:**Jennifer Stanley <jstanle2@uwo.ca>

**CC:**Dr. Qingping Feng <Qingping.Feng@schulich.uwo.ca>

Hi Jennifer,

The lentivirus are in the Lenti-III-HA vector:

<http://www.abmgood.com/viralexplentiIIIHA-dispOne.php?csn=14&ssn=3884&dsn=4023>

The adenovirus are in the Adeno-1 Expression System:

<http://www.abmgood.com/viralexpl/expAdeno1-dispOne.php?csn=14&ssn=3883&dsn=4018>

Let me know if you need additional information. Thanks.

Regards,  
Carmen



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## Viral Expression

### Lenti-III/HA Vector

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Product	Quantity	Kit Catalog Number				Individual Cat. No.	Price
		G327	G328	LV098	LV099		
Lenti-III-HA Tag Vector	10ug	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>			LV022	\$425.00 <input type="checkbox"/>
Lenti-Packaging Mix	100ug	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		LV003	\$225.00 <input type="checkbox"/>
Lentifectin™	1.0ml	<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>		G074	\$135.00 <input type="checkbox"/>
293T Cells	1x10 <sup>6</sup>	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>	LV010	\$150.00 <input type="checkbox"/>
Lenti-EGFP	10ug	<input checked="" type="checkbox"/>			<input checked="" type="checkbox"/>	LV011-a	\$235.00 <input type="checkbox"/>
<b>Kit Price</b>		<b>\$825.00</b>	<b>\$495.00</b>	<b>\$315.00</b>	<b>\$325.00</b>		
		<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		<input type="button" value="Add to Cart"/>

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**Product Details**

**Description**

The Lenti-III-HA expression vector is an improved version of our original Lenti-Easy HA vector, with a greater selection of subcloning sites and a different stable selection marker, Puromycin.

**Advantages of ABM's Lenti-III-HA tag Expression Vectors**

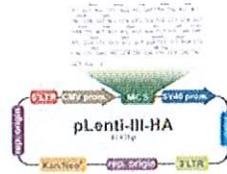
- stable plasmid; rare plasmid DNA re-arrangement
- very high yielding plasmid
- no special competent cells required
- HA-tag compatible
- choice of Puromycin selection marker
- ABM is THE source for your lentiviral expression needs!

User Manual

Infection Protocol

**Vectors**

[pLenti-III-HA](#)



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**Related Products**

Product	Selection Marker	Cat. No.	Price	
GFP Control Lentivirus (10ml)	Neomycin	LV006	\$350	<input type="checkbox"/>
β-Gal Control Lentivirus (10ml)	Neomycin	LV007	\$350	<input type="checkbox"/>
β-Gal Control Lentivector (10ug)	N/A	LV011-b	\$235	<input type="checkbox"/>

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**Notes**

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## Viral Expression

### Adeno-1 Expression System

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Product	Quantity	Kit Catalog Number						Individual Cat. No.	Price	<input type="checkbox"/>
		A100	A110	A120	A097	A098	A099			
Adeno DNA	5ug	✓	✓	✓				A001	\$425.00	<input type="checkbox"/>
pShuttle(+) Vector	5ug	✓	✓					A002	\$155.00	<input type="checkbox"/>
pShuttle(-) Vector	5ug	✓		✓				A003	\$155.00	<input type="checkbox"/>
pShuttle-LacZ Control	5ug	✓	✓	✓				A004	\$45.00	<input type="checkbox"/>
Enzyme Pack: PI-SceI, I-CeuI & Buffers	2x50ul	✓			✓	✓		A005	\$165.00	<input type="checkbox"/>
PCR Screening Set	2x100ul	✓			✓	✓		A006	\$55.00	<input type="checkbox"/>
293 Cells	1x10 <sup>6</sup>					✓		A007	\$150.00	<input type="checkbox"/>
Ad-Competent Cells	10x50ul						✓	A014	\$225.00	<input type="checkbox"/>
Glycogen (20mg/ml)	100ul						✓	A013	\$55.00	<input type="checkbox"/>
<b>Kit Price</b>		<b>\$625.00</b>	<b>\$450.00</b>	<b>\$450.00</b>	<b>\$195.00</b>	<b>\$275.00</b>	<b>\$250.00</b>			

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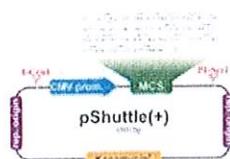
Description

The Adeno-1™ Expression System provides reagents for efficient construction of recombinant adenoviral vectors with a CMV promoter. The principle of Adeno-1™ Expression System is to clone your gene of interest into the adenoviral genome by in vitro ligation (the same as in other subcloning projects). It is quicker, easier, and more reliable for recombinant adenovirus generation than in vivo homologous recombination used in previous approaches. Our subcloning shuttle vectors (pShuttle +/-) have more unique restriction enzyme sites than any other adenoviral cloning system that exists, offering you a variety of choices and simplifying your subcloning manipulation.

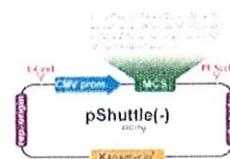
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**Notes**

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----- Original Message -----

**Subject:**Re: Biological Agents Registry Form: Feng lab

**Date:**Mon, 30 Aug 2010 13:35:42 -0700

**From:**Carmen Leung <cleung73@uwo.ca>

**To:**Jennifer Stanley <jstanle2@uwo.ca>

**CC:**Qingping Feng <Qingping.Feng@schulich.uwo.ca>, Sharon Lu  
<Sharon.Lu@schulich.uwo.ca>

Hi Jennifer,

The pCAG-ERT2CreERT2 is a plasmid that will be used to generate a transgenic mouse and will not be used to generate a virus. Therefore, I believe there should not be any biohazard concerns associated with it. If otherwise, please let me know what needs to be addressed regarding use of this material. Thanks.

Sincerely,

Carmen