

Modification Form for Permit BIO-UWO-0089

Permit Holder: Qingping Feng

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

Approved Personnel

(Please stroke out any personnel to be removed)

Yin Liu
 Carmen Leung
 Hoda Moazzen
~~Yan Wu~~
 Murong Liu
 Lily (Fuli) Xiang
~~Ting Zhang~~
 Sharon (Xiangru) Lu

Additional Personnel

(Please list additional personnel here)

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	Adenovirus, Lentivirus, E.coli(055iB5).	
Approved Primary and Established Cells	Rodent [primary]: Mice heart, skin. Human [established]: HEK 293.	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[vector]: adenoviral expression system, lentiviral expression system. [genes]: Cre, Oct3/4, Tbx5, Gata4, Baf60c, Nkx2.5, Nanog, E1A oncogene	LacZ, Stem cell factor (SCF)
Approved Use of Animals	C57BL/6 mice	
Approved Biological Toxin(s)		

Approved Gene Therapy

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Tbx5, Gata4, Raf60c
Nkx2.5, LacZ, SCF.

Approved Plants and Insects

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As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm>

Signature of Permit Holder:

Oris Jones

May 18, 2012

Current Classification: 2+

Containment Level for Added Biohazards:

2

Date of Last Biohazardous Agents Registry Form: Apr 6, 2011

Date of Last Modification (if applicable):

BioSafety Officer(s)*:

*For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.

Chair, Biohazards Subcommittee:

Date:

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?	If plasmids are being used to transfect cells what is the consequence on the eukaryotic cells?

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

** *Please attach a plasmid map.*

****No Material Safety Data Sheet is required for the following strains of E. coli:*

http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

Virus/Plasmid Used for Vector Construction	Vector(s) *	Source of Vector/Plasmid	Gene(s) Transduced/ Transfected	Describe the change that results from transduction/transfection
Adenovirus	Adenovirus	Applied Biological Materials (ABM), Richmond, BC	LacZ, Stem cell factor	Increase gene expression of LacZ and stem cell factor in the heart

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

4.3.1 Will virus be replication defective? YES NO

4.3.2 Will virus be infectious to humans or animals? YES NO

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

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? NO YES

Additional Comments: _____

8.0 Animal Experiments

8.1 Will live animals be used? YES NO If NO, please proceed to section 9.0

8.2 List animal species to be used: **Mice**

8.3 AUS protocol number(s): **2007-011**

8.4 List the location(s) for the animal experimentation and housing: **HS facility, level 2 room**

8.5 Will any of the agents listed in Sections 1-7 be used in live animals

NO YES, specify: **Adenovirus**

8.6 Will the agent(s) be shed by the animal:

YES NO, please justify:

8.7 Indicate the PHAC or CFIA containment level used: 1 2 2+ 3

Permit #: BIO-UWO-0089

Permit Holder: Qingping Feng

Modification: May 18, 2012

Brief Description of the Work – Adenovirus treatment in mice:

Adenoviral constructs Ad-LacZ, Ad-SCF, Ad-Tbx5, Ad-Gata4, Ad-Baf60c and Ad-Nkx2.5 were constructed by Applied Biological Materials (ABM), Vancouver, BC, and are stored in our -80 °C freezer. Adult mice will be anesthetized with intraperitoneal administration of ketamine and xylazine cocktail. Mice will be intubated and ventilated with a respirator. Chest will be opened and the left descending coronary artery will be occluded with a suture. Immediately after coronary artery occlusion, single adenoviral construct or 2-3 adenoviral constructs in combination (total titer 10^9 pfu per mouse) will be injected into the peri-infarct area of the heart. Chest will then be closed and animals are allowed to recover. Following surgery and treatment of adenovirus, animals will be housed in filtered cages. Mice will be sacrificed at day 5 or day 30 after surgery.

All procedures will be done in the HS facility level 2 room. To minimize health risk, the operator will wear a protective gown, gloves, glasses, and a fit-tested N95 respirator. Cage changes after the surgery will be done in a biological safety cabinet.

Hi Jennifer,

In this experiment, the chest will be open and mice will be ventilated with a respirator. Since the respirator and the operating microscope can not fit into a biosafety hood, we have to do the surgeries and adenovirus treatment outside the biosafety hood. However, after the animals are recovered, cage changes will be done in a biosafety hood. As we discussed yesterday, the operator will wear a fit-tested N95 respirator. I hope this will solve the problem.

The revised description is attached. There are no changes to other documents.
Thanks.

Qingping

Material Safety Data Sheet



Stratagene pFB-Neo-LacZ Control Vector, Catalog #240029

1. Product and company identification

Product name	: Stratagene pFB-Neo-LacZ Control Vector, Catalog #240029
Material uses	: Analytical reagent. 0.01 ml
Supplier/Manufacturer	: Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Part No.	: 240029
Validation date	: 04/13/2011
<u>In case of emergency</u>	: 1-800-894-1304

2. Hazards identification

Physical state	: Liquid.
OSHA/HCS status	: While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.

Emergency overview

Hazard statements : NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

Precautions : No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.

Potential acute health effects

Inhalation : No known significant effects or critical hazards.

Ingestion : No known significant effects or critical hazards.

Skin : No known significant effects or critical hazards.

Eyes : No known significant effects or critical hazards.

Potential chronic health effects

Chronic effects : No known significant effects or critical hazards.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation : No specific data.

Ingestion : No specific data.

Skin : No specific data.

Eyes : No specific data.

Medical conditions aggravated by over-exposure : None known.

See toxicological information (Section 11)

3. Composition/information on ingredients

Name	CAS number	%
No hazardous ingredient		

There are no ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4. First aid measures

- Eye contact** : Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.
- Skin contact** : In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if symptoms occur.
- Inhalation** : Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms occur.
- Ingestion** : Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms occur.
- Protection of first-aiders** : No action shall be taken involving any personal risk or without suitable training.
- Notes to physician** : No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

5. Fire-fighting measures

- Flammability of the product** : In a fire or if heated, a pressure increase will occur and the container may burst.
- Extinguishing media**
- Suitable** : Use an extinguishing agent suitable for the surrounding fire.
- Not suitable** : None known.
- Special exposure hazards** : No action shall be taken involving any personal risk or without suitable training.
- Hazardous thermal decomposition products** : No specific data.
- Special protective equipment for fire-fighters** : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

6. Accidental release measures

- Personal precautions** : No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Put on appropriate personal protective equipment (see Section 8).
- Environmental precautions** : Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
- Methods for cleaning up** : Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble. Alternatively, or if water-insoluble, absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7. Handling and storage

- Handling** : Put on appropriate personal protective equipment (see Section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas.
- Storage** : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8. Exposure controls/personal protection

Ingredient

No exposure limit value known.

Consult local authorities for acceptable exposure limits.

- Recommended monitoring procedures** : If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.
- Engineering measures** : No special ventilation requirements. Good general ventilation should be sufficient to control worker exposure to airborne contaminants. If this product contains ingredients with exposure limits, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure below any recommended or statutory limits.
- Hygiene measures** : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal protection

- Respiratory** : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
- Hands** : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
- Eyes** : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.
- Skin** : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
- Environmental exposure controls** : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.
- Other protection** : Not available.

9. Physical and chemical properties

Physical state	: Liquid.
Flash point	: Not available.
Auto-ignition temperature	: Not available.
Flammable limits	: Not available.
Color	: Not available.
Odor	: Not available.
pH	: 7.5
Boiling/condensation point	: 100°C (212°F)
Melting/freezing point	: 0°C (32°F)
Density	: Not available.
Vapor pressure	: Not available.
Vapor density	: Not available.
Odor threshold	: Not available.
Evaporation rate	: Not available.
Solubility	: Easily soluble in the following materials: cold water and hot water.

10. Stability and reactivity

Chemical stability	: The product is stable.
Conditions to avoid	: No specific data.
Materials to avoid	: Reactive or incompatible with the following materials: oxidizing materials, reducing materials, metals, acids and alkalis.
Hazardous decomposition products	: Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Possibility of hazardous reactions	: Under normal conditions of storage and use, hazardous reactions will not occur.

11. Toxicological information

Acute toxicity

Not available.

Irritation/Corrosion

Conclusion/Summary : Not available.

Sensitizer

Conclusion/Summary : Not available.

Chronic toxicity / Carcinogenicity / Mutagenicity / Teratogenicity / Reproductive toxicity

Not available.

12. Ecological information

Ecotoxicity	: No known significant effects or critical hazards.
Other adverse effects	: No known significant effects or critical hazards.

13. Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Significant quantities of waste product residues should not be disposed of via the foul sewer but processed in a suitable effluent treatment plant. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14. Transport information

Regulatory information

DOT / IMDG / IATA / : Not regulated.

15. Regulatory information

HCS Classification : Not regulated.

U.S. Federal regulations : **TSCA 8(a) IUR:** Partial exemption

United States inventory (TSCA 8b): All components are listed or exempted.

SARA 302/304/311/312 extremely hazardous substances: No products were found.

SARA 302/304 emergency planning and notification: No products were found.

SARA 302/304/311/312 hazardous chemicals: No products were found.

SARA 311/312 MSDS distribution - chemical inventory - hazard identification: No products were found.

Clean Water Act (CWA) 311: Edetic acid

Clean Air Act Section 112(b) Hazardous Air Pollutants (HAPs) : Not listed

Clean Air Act Section 602 Class I Substances : Not listed

Clean Air Act Section 602 Class II Substances : Not listed

DEA List I Chemicals (Precursor Chemicals) : Not listed

DEA List II Chemicals (Essential Chemicals) : Not listed

State regulations

Massachusetts : None of the components are listed.

New York : None of the components are listed.

New Jersey : None of the components are listed.

15. Regulatory information

Pennsylvania : None of the components are listed.

California Prop. 65

No products were found.

16. Other information

Label requirements : NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

Date of issue : 04/13/2011

Date of previous issue : No previous validation.

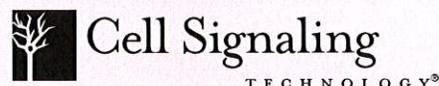
Version : 1

 Indicates information that has changed from previously issued version.

Notice to reader

Disclaimer: The information contained in this document is based on Agilent's state of knowledge at the time of preparation. No warranty as to its accurateness, completeness or suitability for a particular purpose is expressed or implied.

Material Safety Data Sheet (MSDS) for Human Stem Cell Factor (hSCF)



I. Identification:

Product name: Human Stem Cell Factor (hSCF)
Product Catalog: 8925
CAS#: None
Manufacturer Supplier: Cell Signaling Technology
 3 Trask Lane
 Danvers, MA 01923 USA
 978-867-2300 TEL
 978-867-2400 FAX
 978-578-6737 EMERGENCY TEL

II. Composition/Information:

This product is a lyophilized mixture of substances. According to 29 CFR 1910.1200(d), mixtures with hazardous ingredients at less than <1% and carcinogens at less than <0.1% are considered non-hazardous.

Substance Name: Human Stem Cell Factor, recombinant

Ingredients:	Carrier-Free	With Carrier	CAS#
Human Stem Cell Factor, hSCF	98%	5%	none
Bovine serum albumin	0%	95%	9048-46-8

III. Hazard Identification:

This product is not for use in humans. It is intended for research purposes only.
 To the best of our knowledge, the chemical, physical, and toxicological properties of this material have not been established.

EMERGENCY OVERVIEW: Target organs: Blood, bone marrow. No known hazards.

HMIS	Health: 0	Flammability: 0	Reactivity: 0
NFPA	Health: 0	Flammability: 0	Reactivity: 0

IV. First Aid Measures:

Inhalation: If inhaled, remove to fresh air. If breathing is difficult, get medical attention.
Ingestion: If swallowed, wash out mouth with water provided person is conscious. Get medical attention.
Skin exposure: In case of contact, immediately wash skin with soap and water for at least 15 minutes. Remove contaminated clothing. Wash clothing before reuse.
Eye exposure: In case of contact with eyes, immediately flush eyes with water for at least 15 minutes. Get medical attention.

V. Fire Fighting Measures:

Flash Point: Data not available.
Autoignition Temperature: Data not available.
Explosion: Data not available.
Fire extinguishing media: Water spray, dry chemical, alcohol foam, or carbon dioxide.
Firefighting: Wear protective clothing and self-contained breathing apparatus to prevent contact with skin and eyes. May emit toxic fumes under fire conditions.

VI. Accidental Release Measures: Wear appropriate personal protective equipment. Sweep up material and avoid raising dust. Transfer to a closed chemical waste container for disposal. Wash spill site after material has been picked up for disposal.

VII. Handling And Storage:

Store in tightly closed container at -20°C. Avoid inhalation. Avoid contact with eyes, skin, and clothing. Wash thoroughly after handling.

VIII. Exposure Controls/Personal:

Ventilation System: A system of local and/or general exhaust is recommended.
Skin Protection: Wear compatible chemical resistant gloves and protective clothing.
Eye protection: Wear protective safety glasses or chemical safety goggles. Maintain eye wash fountain and quick-drench facilities in work area.

IX. Physical And Chemical Properties

Appearance: lyophilized powder
pH: data not available
Melting Point: data not available
Boiling Point: data not available
Freezing Point: data not available
Volatile Organic Compounds: data not available
Solubility in water: soluble in phosphate buffered saline

X. Stability and Reactivity:

Stability: Stable under normal conditions.
Conditions/materials to avoid: Data not available
Hazardous Decomposition: No data available.

XI. Toxicological Information:

Acute Effects: Data not available
Chronic Effects: Data not available
Potential Health Effects: Not established.
Inhalation: May be harmful, may be irritating to mucous membranes and upper respiratory tract.
Skin: May be harmful if absorbed through skin. May cause skin irritation.
Eyes: May cause eye irritation.
Ingestion: May be harmful if swallowed.

XII. Ecological Information: No data available.

XIII. Disposal Considerations: Dispose of in accordance with federal, state, local environmental regulations.

XIV. Transport Information:

DOT: This substance is considered Non-Hazardous for transport.
IATA: This substance is considered Non-Hazardous for air transport.

XV. Regulatory Information:

EU Regulations/Classifications/Labeling Information: None.
US Regulatory Information:
SARA Listed: No.
Canada (WHMIS): DSL No, NDSL No.

XVI. Other Information:

This compound is sold only for research use only. It is not for use in humans. To the best of our knowledge, this document is accurate. It is intended to serve as a guide for safe use of this product in a laboratory setting by experienced personnel. The burden of safe use of this material rests entirely with the user. Cell Signaling Technology, Inc., shall not be held liable for any damage resulting from the handling of or from contact with the above product.

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: July 9, 2010
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 (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, 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 Biohazards 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	<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:

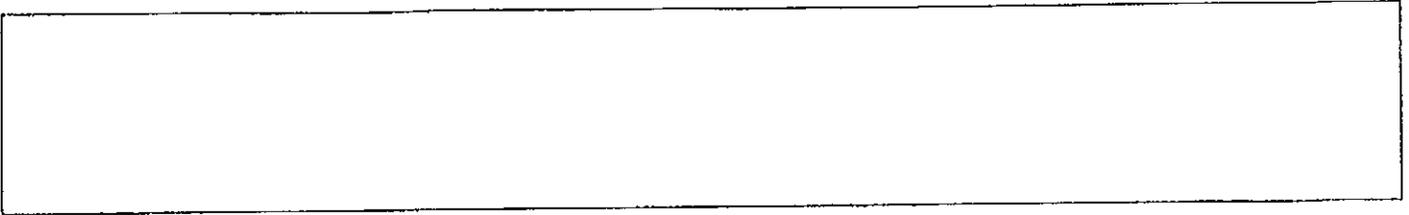
<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Sharon Lu	Sharon.lu@schulich.uwo.ca	July 14, 2008
Lily Xiang	Fxiang2@uwo.ca	May 26, 2008
Yin Liu	Yliu258@uwo.ca	Registered for refresher training
Ting Zhang	Tzhang53@uwo.ca	May 26, 2008
Carmen Leung	Cleung73@uwo.ca	Registered for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

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% CO₂ 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. 2.5×10^7 E. coli bacteria/ g body weight of the mouse 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

Department of Physiology and Pharmacology, University of Western Ontario

London, Ontario, Canada

Qingping.Feng@schulich.uwo.ca

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:

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	<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 checked="" type="radio"/> 2 <input 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)

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	Cre (not oncogenic gene) Catalogue #: 000198A	Gene knockdown of specifically floxed genes; cells remain healthy and do not turn cancerous.
Lentivirus	Lentivirus expression system	ABM	Oct 4 (Cat. #: LV010061), Tbx5 (Cat. #: LV010060), Gata4 (Cat. #: LV010058), Baf60c (LV010062), Nkx2.5 (Cat. #: LV010059), Nanog (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 ^{as} ~~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

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 blood 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 LD₅₀ (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

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:

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:

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: _____

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? YES NO
If YES, Please attach the CFIA permit & 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 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

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 Jones* Date: March 24, 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: *[Signature]*
Date: Apr. 6 2011

2) Safety Officer for the University of Western Ontario SIGNATURE: *J Stanley*
Date: April 4, 2011

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

Approval Number: BIO-UWO-0089 Expiry Date (3 years from Approval): April 5, 2014

Special Conditions of Approval:

- Waste can be autoclaved or decontaminated.
- Follow attached Workplace Health Policy (Hepatitis based vectors)



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)

Date:Thu, 24 Mar 2011 11:58:03 -0400

From:Carmen Leung <cleung73@uwo.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

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

Hi Jennifer,

Please find attached an updated version of the Feng lab biological agents registry form with the requested changes.

Regarding Section 4.4, I am unsure what you mean by updating this section to reflect the use of HEK cells since this section does not ask about the use of HEK cells. Please clarify and I will be happy to answer any questions regarding this.

To confirm section 6.0, yes, we will be injecting lentivirus into live animals as well as cells.

Regards,

Carmen

----- 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



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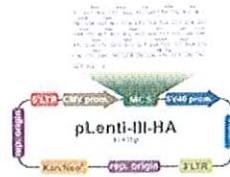
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