

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

Approved Personnel

(Please stroke out any personnel to be removed)

Danielle Lapierre
Mao Jiang
Souzan Armstrong
Tom Chrones

Additional Personnel

(Please list additional personnel here)

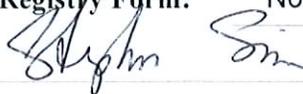
	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E.coli, Recombinant Adenovirus	
Approved Cells	Human (primary), colon esophagus, Rodent (primary), Human (established) HEK 293, Rodent (established) RAW 264.7, CHO, Non-human primate (established) COS,	
Approved Use of Human Source Material	tissues (unpreserved), esophagus, colon	
Approved GMO	SV 40 Large T antigen, COS, Adenovirus, pcDNA3, pEGFP, pE4FP, pAd/CMV/VS-DEST	Plasmid EGFP-LC3
Approved use of Animals	rabbits, guinea pigs, rats mice	

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder  Feb 17 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

Approved Toxin(s)

Tetrodo toxin, Botulinum toxin, Pertussis toxin

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

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Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder

Stephen Sims Feb 17 2009

BioSafety Officer(s):

Chair, Biohazards Subcommittee:



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EGFP-LC3

Insert: EGFP-LC3B, NM_022818

Description: H. sapiens (human), EGFP, Neomycin

Article: PLoS Biol. 2005 May . 3(5):e156.

Search Summary
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Also See
Karla Kirkegaard Lab Plasmids

1st. We will receive the plasmid (which is a small strand of DNA that encodes for the production of a specific protein PLUS the Green Fluorescent Protein (GFP) marker) and will replicate it using bacterial culture.

2nd. After enough of the plasmid has been replicated we will use it to transfect bone cells (osteoclast-like and osteoblasts in culture). Transfection pretty much involves the addition of the plasmid to the cells in culture in order for the plasmid DNA to enter the cells and replicate. The GFP tag allows us to monitor the protein expression.

That is all.

Let me know if you need more detailed information.

Thanks,

Tom

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Additional Personnel

(Please list additional personnel here)

Approved Microorganisms

Please stroke out any approved
Biohazards to be removed below

E.coli, Recombinant Adenovirus

Write additional Biohazards for
approval below. *

Approved Cells

Human (primary), colon esophagus, Rodent
(primary), Human (established) HEK 293,
Rodent (established) RAW 264.7, CHO, Non-
human primate (established) COS,

Approved Use of Human Source Material

tissues (unpreserved), esophagus, colon

Approved GMO

SV 40 Large T antigen, COS, Adenovirus,
pcDNA3, pEGFP, pE4FP, pAd/CMV/VS-
DEST

Approved use of Animals

rabbits, guinea pigs

Rats, Mice

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder: Stephen Sims May 26 2008

BioSafety Officer(s): Stanley June 27/08

Chair, Biohazards Subcommittee: G.M. Koller 2 July '08

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

Approved Toxin(s)

Tetrodo toxin, Botulinum toxin

Pertussis toxin

- Pertussis toxin is used to study cell signalling pathways.
- The toxin targets a specific G protein, which is involved in signalling for ~~sy~~ certain receptors.
- We purchased the smallest amount available, and will handle it only in the biosafety cabinet.
- 25 mm culture dishes containing cells will be treated with pertussis toxin and maintained in a culture incubator for 4 hours.
- Coverslips with treated cells will be removed from the incubator, washed, and viewed on a microscope.
- Residual solution containing pertussis toxin will be mixed with bleach, in accordance with existing practices.

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder: Stephen Sims May 26 2008

BioSafety Officer(s): Jen Stanley June 27/08

Chair, Biohazards Subcommittee: G.M. Kildor 2 July 08

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Stephen M. Sims
SIGNATURE *Stephen M. Sims*
DEPARTMENT Physiology & Pharmacology
ADDRESS DSB 0073 - uwo
PHONE NUMBER 661-3768
EMAIL stephen.sims@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 0074
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S): Please See Attached

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES _____

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

- i) Tom Chronos
- ii) Mao Jiang
- iii) Suzan Armstrong
- iv) Danielle Lapierre
- v) Jason Kovac

1.0 Microorganisms

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

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?
<i>E. Coli</i>	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	1500 mL
Recombinant Adenovirus non-replicating	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	2 x T75 flasks
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required.

1 (2) 3

1.4 Source of microorganism(s) or biological agent(s)? Invitrogen

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 (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Colon, Esophagus, obtained from surgical specimens
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Rats & Mice (Bone and Bone marrow)
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No	Rect (penis tissue) All
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	HEK 293	Invitrogen, ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	RAW 264.7, CHO	ATCC
Non-human primate	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	COS	ATCC
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No		

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 (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 if the following will be used in the laboratory

◆ Human blood (whole) or other bodily fluids	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	If YES, Specify _____
◆ Human blood (fraction) or other bodily fluids	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	If YES, Specify _____
◆ Human organs (unpreserved)	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	If YES, Specify _____
◆ Human tissues (unpreserved)	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	If YES, Specify <u>Esophagus, colon</u>

3.3 Is human source known to be infected with and infectious agent YES NO
 If YES, please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

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 sequences from the following be involved:

◆ HIV	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
if YES specify _____		
◆ HTLV 1 or 2 or genes from any CDC class 1 pathogens	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
if YES specify _____		
◆ Other human or animal pathogen and or their toxins	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
if YES specify _____		

4.3 Will intact genetic sequences be used from

◆ SV 40 Large T antigen	<input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	If YES specify <u>CDS</u>
◆ Known oncogenes	<input type="checkbox"/> YES <input checked="" type="checkbox"/> NO	If YES specify _____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction YES NO
 If YES name virus Adenovirus, non replicating

4.5 List specific vector(s) to be used: pCDNA3, pEGFP, pEYFP, pAd/CMV/V5-DEST
derivatives of (Inventrogen)

4.6 Will virus be replication defective YES NO

4.7 Will virus be infectious to humans or animals YES NO

4.8 Will this be expected to increase the Containment Level required YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? YES NO

5.3 How will the virus 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 NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? YES NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used rabbits, guinea pigs → AUS protocol

6.3 AUS protocol # _____

6.4 If using murine cell lines, have they been tested for murine pathogens? YES NO

7.0 Use of Animal species with Zoonotic Hazards

- 7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:
- Pound source dogs YES NO
 - Pound source cats YES NO
 - Sheep or goats YES NO
 - Non- Human Primates YES NO If YES specify species _____
 - Wild caught animals YES NO If YES specify species _____
colony # _____

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 Tetrodotoxin + Botulinum Toxin

8.3 What is the LD₅₀ (specify species) of the toxin
Tetrodotoxin LD₅₀ for Humans is 5-30 mg/kg
Botulinum Toxin for Humans is 3 ng/kg

* Must keep less than 5mg of each toxin as
* Must store in locked facility / freezer. QP

9.0 Import Requirements

9.1 Will the agent be imported? YES NO
If no, please proceed to Section 10.0
If yes, country of origin _____

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

9.3 Has an import permit been obtained from CFIA for animal pathogens? YES NO

9.4 Has the import permit been sent to OHS? YES NO
If yes, Permit # _____

10.0 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

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

SIGNATURE *Stephen Sinn* Oct 17 2007

11.0 Containment Levels

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

11.2 Has the facility been certified by OHS for this level of containment? YES NO

11.3 If yes, please give the date and permit number: BIO-UWO-0096 a1

12.0 Approvals

UWO Biohazard Subcommittee

Signature *G.M. Kiddle* Date 22 Nov. '07

Safety Officer for Institution where experiments will take place

Signature *Jennifer Harley* Date Nov 22 / 07

Safety Officer for University of Western Ontario (if different than above)

Signature _____ Date _____