Modification Form for Permit BIO-UWO-0209

Permit Holder: Robert Cumming

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

~~Tammy Lewis~~

Additional Personnel
(Please list additional personnel here)

Jordanna Newington
Kyle Dailey
Robert Arseneault

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E. coli DH5 alpha	
Approved Primary and Established Cells	Human (established) U87-MG, Rodent (established) NIH 3T3, PC12, HT22	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	SV 40 Large T antigen already inserted in HT22 cell, pcDNA 3.1 plasmid	pcDNA3-Myc3-Nrf2 pcDNA3-EGFP-C4-Nrf2 pcDNA3-HA2-Keap1 pCAG-mGFP-Actin
Approved Use of Animals		
Approved Biological Toxin(s)		

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

Signature of Permit Holder Robert Cumming

Current Classification: 2 Containment Level for Added Biohazards: 2

Date of Last Biohazardous Agents Registry Form: May 8, 2008

Date of Last Modification (if applicable):

BioSafety Officer(s): Jennifer Stanley

Chair, Biohazards Subcommittee: Date:

Modification Form for Permit BIO-UWO-0209
Permit Holder: Robert Cumming
May 24, 2011

Description of work that biohazards will be used for

The following new plasmids will be used:

pcDNA-Myc3-Nrf2
pcDNA3-EGFP-C4-Nrf2
pcDNA3-HA2-Keap1
pCAG-mGFP-Actin

All plasmids are based on the pcDNA 3.3 backbone from Invitrogen (MSDS sheet on file with Biosafety Office).
The genes within these plasmids are non-toxic, non-tumorgenic and non-transforming when expressed in mammalian cells. The above plasmids will be transfected (using lipofectamine) into PC12 and HT22 cultured nerve cell lines for phenotypic analysis.

Storage and disposal

The plasmids will be stored in my -80°C freezer until use. The plasmids will be propagated in DH5α bacteria and frozen glycerol stocks will also be maintained in my -80°C freezer. All plasmid containing bacteria will either be treated with bleach overnight (liquid cultures) or autoclaved (soft agar cultures) before disposal.

Bio - UWO - 0209

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: September, 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 Robert Cumming
SIGNATURE Robert Cumming
DEPARTMENT Biology
ADDRESS BGS, Room 3078
PHONE NUMBER 519-661-2111, ext 81578
EMAIL rcummin5@uwo.ca

Location of experimental work to be carried out: Building(s) BGS Room(s) 30

*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.

GRANT TITLE(S):
① Subcellular analysis of the disulfide proteome in mammalian cells
② GAPDH and related proteins as therapeutic targets in HD

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NSERC, HighQ Foundation

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

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

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☑ YES O 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?	Source/ Supplier	Health Canada or CFIA Containment Level
DH5α bacteria	O Yes ☑ No	O Yes ☑ No	O Yes O No	100 ml	Invitrogen	☑ 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	O Yes ☑ No	
Rodent	O Yes ☑ No	
Non-human primate	O Yes ☑ No	
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	containment level
Human	☑ Yes O No	U87-MG level 2	The Salk Institute	Level 1
Rodent	☑ Yes O No	NIH3T3, PC12, HT22	The Salk Institute	Level 1
Non-human primate	O Yes O No			
Other (specify)	O Yes O No			

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

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

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

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level** (select one)
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs (preserved)		O Yes O No		O 1 O 2 O 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 O NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

- HIV O YES ☑ NO
  if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens O YES ☑ NO
  if YES specify_____
- Other human or animal pathogen and or their toxins O YES ☑ NO
  if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☑ YES O NO If YES specify Already inserted into HT22 cell genome
- Known oncogenes O YES ☑ NO If YES specify_____

4.4 Will a live viral vector(s) or bacterial plasmid be used for gene transduction ☑ YES O NO
If YES name pcDNA 3.1 plasmid.
Please attach a Material Safety Data Sheet or equivalent.

4.5 List specific vector(s) to be used: pcDNA 3.1 (Invitrogen)

4.6 Will virus be replication defective O YES ☑ NO

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

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? O 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? O YES ✓ NO

5.3 How will the virus be administered? N/A

5.4 Please give the Health Care Facility where the clinical trial will be conducted: N/A

5.5 Has human ethics approval been obtained? O YES ✓ NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 If using murine cell lines, have they been tested for murine pathogens? O 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 O YES ✓ NO
- Pound source cats O YES ✓ NO
- Cattle, sheep or goats O YES ✓ NO
- Non- Human Primates O YES ✓ NO If YES specify species _____
- Wild caught animals O YES ✓ NO If YES specify species _____ colony # _____
- Birds O YES ✓ NO
- Others (wild or domestic) O YES ✓ NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin_____

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

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

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

9.0 Import Requirements

9.1 Will the agent be imported? O 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? O YES O NO

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

9.4 Has the import permit been sent to OHS? O YES O 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
- Employee Health and Safety Orientatioin

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 Robert Cumming

11.0 Containment Levels

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

11.2 Has the facility been certified by OHS for this level of containment? ✓ YES O NO Not Yet

11.3 If yes, please give the date and permit number: May 7, 2008, BIO-UWO-02092L

12.0 Approvals

UWO Biohazard Subcommittee

Signature G. M. Gilder Date 8 May '08

Safety Officer for Institution where experiments will take place

Signature J Stanley Date May 7, 2008

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

Signature_____ Date_____

Expiry Date (3 years from Approval): May 8, 2011

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