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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR: KATHLEEN A HILL
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Location of experimental work to be carried out: Building(s) WSC Room(s) 329

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

FUNDING AGENCY/AGENCIES: CNIB NSERC* KRA*
GRANT TITLE(S): SPONTANEOUS HOTSPOT MUTATIONS* DRUG FLOW IN THE EYE

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

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

ANITA PATENAUDE*	WSC 329
TOM MACPHERSON	
ALEX LALIBERTE	
ANDREW POOTHILLIL	
MATT EDWARDS	
ALEX YAN	
CHRIS IBBOTSON	
~~SARAH HUNNAN~~	

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ○ NO
(including but not limited to 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
Ecoli 3555	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500ml – 3L	STRATAGENE	☒ 1 ○ 2 ○ 3
Ecoli G1250	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500ml	STRATAGENE	☒ 1 ○ 2 ○ 3
λ phage	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	10^8 particles	STRATAGENE	☒ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 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	○ Yes ○ No		Not applicable
Rodent	○ Yes ○ No		
Non-human primate	○ Yes ○ No		
Other (specify)	○ Yes ○ No		

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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	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES O 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)**	**PHAC 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 or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (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? O YES O NO If no, please proceed to Section 5.0

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

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

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments Biohards are not exposed to animals

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

6.2 Name of animal species to be used Mus musculus Rabbit New Zealand White

6.3 AUS protocol # 2009-30 & 32-33

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ X NO

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7.0 Use of Animal species with Zoonotic Hazards

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

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

EYES - ABBATTOIR SOURCE PIG COW RABBIT

8.0 Biological Toxins

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

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

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

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

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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10.0 Plants Requiring CFIA Permits

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

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

10.3 What is the origin of the plant? _____

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

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

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

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

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

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

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

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

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SIGNATURE _____

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, permit # if on-campus BIO UWO 0110
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: May 24 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

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

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

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

Special Conditions of Approval:

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Hill BIO UWO 0110

Cadaveric Eye Research

Omnipaque™ CT contrast agent and Gold-Fluorescien tagged VEGF inhibitor will be injected midvitreous into cadaveric bovine, lapine and porcine eyes (Abbattoir supplier). After contrast or tagged antibody injection, eyes will be transferred to a microCT scanner where scans will be conducted at prescribed intervals over the course of 8 hours to monitor contrast motion (to the point that the contrast agent or tagged antibody has reached the retina). CT scans will be analyzed to model agent flow through the eye using the advanced finite element package ABAQUS for computational fluid dynamics (CFD) simulations. Drug concentration profiles from our CFD models will be correlated to experimental data to determine percentage error on a voxel per voxel basis as a function of time. Once simulations are created and experimentally verified *in vivo*, extrapolation to a human ocular geometry will produce a tool to study topical drug delivery in glaucoma.

Hill Lab WSC 329

Transgenic mouse mutation detection assay – use of E. coli and lambda phage

cII mutant detection

Samples of frozen skin tissue (200 mg) were used to extract high molecular weight genomic DNA using a standard phenol/chloroform protocol for the Big Blue® Assay (23). Isolated DNA samples were dissolved in TE buffer (pH 7.5), brought to an optimal concentration (0.5 μg/μL) and stored at 4°C. The *cII* transgenes were recovered and packaged *in vitro* using Transpak and the λ Select-*cII*™ protocol. Lambda phages were used to infect cultures of *E. coli* strain G1250, which were plated on 9-cm diameter plates and grown under selective (24°C, 48 hours) or control (37°C, 24 hours) conditions (24). Putative *cII* mutants were cored and stored at 4°C in 500 μL of SM buffer with 5 μL of chloroform. These putative mutants were cored and used to infect *E. coli* G1250 cultures to confirm the mutant phenotype by replating under the selective conditions (24). Selective conditions were monitored by the failure of wild type phage to produce plaques. Mutants with confirmed phenotype were cored and placed in 25 μL of sterile dH_2O and stored at -20°C.

cII mutation identification

The *cII* sequence from every replated mutant was sequenced following PCR amplification and purification. A 475 base pair sequence (5' bp 38248 to 3' bp 38723; base number is according to (25)) containing the entire *cII* gene and flanking regions was amplified by PCR with the *cII* primers and genotyping PCR conditions previously mentioned. The amplicon was purified using 0.1 μL of Exonuclease I (20 units/ μL; New

England Biolabs, Ipswich, MA), 2 µL rAPid Alkaline Phosphatase (1 unit/µL; Roche Applied Science, Basel, Switzerland), and 4 µL water per 6 µL of PCR product. The reaction consisted of: incubation for 1 hour at 37°C and inactivation at 75°C for 15 minutes. The *cII* gene was sequenced using a BigDye® v3.1 Cycle sequencing Kit (Applied Biosystems) and centriSep 8 column for purification (Princeton Separations, Adelphia, NK) in either the forward (5'-AAAAAGGGCATCAAATTAAACC -3') or reverse (5'-CCGAAGTTGAGTATTTTTGCTGT-3') direction using an ABI automated 3130xl Genetic Analyzer (Applied Biosystems). Every twentieth sample was reamplified and resequenced to confirm the sample origin of the mutation. Several mutants were PCR amplified from the original cored plaque to confirm the mutation identified. Sequence chromatograms were analyzed in a blinded fashion by two individuals using SeqScape (Applied Biosystems, Foster City, CA) and Sequencer software (Gene Codes, Ann Arbor, MI). The mutant sequences were compared to the published wild type *cII* sequence (Accession #J02459).

Big Blue *lacI* assay (use of *E. coli* SCS-8)

In Vitro Packaging and Plating of λ Concatamer DNA

Big Blue λ DNA concatamers produced in *E. coli SCS-8* and genomic DNA from a Big Blue mouse were packaged using Transpack® packaging extract (Stratagene, La Jolla, CA). Big Blue mouse DNA with a known high packaging efficiency (>100,000 pfu/reaction) was packaged as a positive control. The efficiency of each packaging reaction was estimated. If a sufficient number of pfu was present in the each of the packaging reactions, the DNA was packaged again for plating and mutant detection.

Packaged λ phage particles from Big Blue mouse DNA and from 12- and 18-minute Big Blue λ DNA concatamer preparations were plated on Big Blue bottom agar with 5-Bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal; Stratagene) in 25 x 25 cm assay trays (Stratagene) the day after *in vitro* packaging. Three independent observers identified the colour intensity and morphology of each colour control mutant and mutants recovered. Mutant plaques were visualized on a red plexiglass screen, which increased the sensitivity of the detection of light blue plaques. They were classified based on morphology and blue intensity {Nishino, 1996 #146;Rogers, 1995 #127}. Mutant plaques were classified morphologically as circular, sectored, pinpoint, or non-circular {Nishino, 1996 #146}. Standard colour mutant phage particles containing known mutations in the *lacI* gene were used for comparison of blue mutant plaque colour intensity {Farabaugh, 1978 #14;Rogers, 1995 #127}. The known mutations and blue colour intensities were: CM0 (C→ T at base position 530, very light); CM1 (C→ A at base position 179, light); CM2 (G→ A at base position 381, medium); and CM3 (C→ T at base position 977, dark). All mutant plaques were cored, given a unique sample identifier, and stored at 4°C in 500 µl SM with 50 µl chloroform.

Mutant Plaque Isolation and PCR Amplification of lacI

Each mutant plaque was replated at low density on Big Blue bottom agar containing X-gal to confirm the mutant phenotype, identify false positives, and separate mutant plaques from wild type plaques to obtain clones of a single copy of *lacI* for DNA sequencing. Replating was considered successful if there were more than two well-isolated mutant plaques of the same colour intensity in a 9 cm Petri dish with no

crowding. Well-isolated mutant plaques that were blue upon replating were cored, resuspended in 100 µl TE, boiled for 5 minutes, and stored at -20°C.