THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 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	Priti Krishna
DEPARTMENT	Biology
ADDRESS	B&G Sci Rm 3061
PHONE NUMBER	Ext 86473
EMERGENCY PHONE NUMBER(S)	519-438-9518
EMAIL	pkrishna@uwo.ca

Location of experimental work to be carried out: Building(s) B&G Sci Bldg Room(s) 3061

*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: NSERC, AAFC-ABIP, OMAFRA
GRANT TITLE(S):
Functions of Hsp90,
Brassiosteroid mediated stress tolerance
Sea buckthorn transcriptome and metabolome

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

Name	UWO E-mail Address	Date of Biosafety Training
Jason Lehrer	jlehrer@uwo.ca	Feb 20th, 2009
Maryam Khodai-Kalaiki	mkhodaik@uwo.ca	
Bishun Prasad	bprasad2@uwo.ca	Nov 17th 2010
Tawhid Rahman	trahman3@uwo.ca	Dec 7th 2010
Tahira Fatima	tfatima@uwo.ca	March 2009
Sangita Sahni	ssahani3@uwo.ca	Nov 17th, 2010
Rajesh gupta	g_rkumar2000@yahoo.co.in	

See E-mail

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.

1. *E.coli* (DH5α) used for cloning, disposed by autoclaving
2. Arabidopsis and *Brassica napus* transgenic plants (used for research only to understand functional significance of gene(s), disposed by autoclaving.
3. Ethidium Bromide, used in DNA and RNA visualization, disposed by chemical waste disposal through RPR Environmental.
4. Acrylamide: Used in Protein Gel electrophoresis, , disposed by chemical waste disposal through RPR Environmental.

Please include a one page research summary or teaching protocol.

The highly conserved and abundant molecular chaperone Hsp90 (heat shock protein 90) plays key roles in signal transduction, protein degradation and trafficking, chromatin remodeling, epigenetic regulation, and morphological evolution. The significance of Hsp90 is indicated by its 1) **indispensable role in** regulating cell growth through modulations of various signal transduction pathways and its participation in protecting cells from stress; 2) **potential as a capacitor for morphological evolution,** linking response to environmental stresses with development in a way that could influence evolutionary change; 3) **position as a cancer chemotherapeutic target** due to its interactions with key oncogenic client proteins. Hsp90 inhibitors like geldanamycin (GA) and radicicol (RA) interrupt the intrinsic ATPase activity of Hsp90, causing degradation of the client proteins via the ubiquitin-proteasome pathway.

The current project is focused on obtaining a detailed understanding of the functions of Hsp90 in plants and its mode of action. Hsp90 is of particular interest in plants because plants are subjected to sudden changes in their environment that invoke rapid molecular responses, the Hsp90 protein family is the largest in plants as compared to other orgnisms, and plants contain an additional compartment, the plastid. The study of Hsp90 functions in plants will undoubtedly lead to novel aspects of Hsp90, advancing the link between stress, development and evolution. A good knowledge of the plant Hsp90 system will facilitate our understanding of how proteins are folded, stabilized, activated and trafficked in plant cells.

In recent years we have identified components of the ethylene signaling pathway, ethylene receptors ERS2 and EIN4, and the negative regulator CTR1) as potential novel client proteins of Hsp90. In addition we have uncovered by an in silico approach 24 novel tetratricopeptide repeat (TPR) domain proteins in Arabidopsis as novel co-chaperones of plant Hsp90. This first ever near to complete set of potential TPR co-chaperones uncovered in any organism suggests that the Hsp90 chaperone machinery relies on TPR co-chaperones more than it was previously realized.

The objectives of this project are to 1) characterize in detail Hsp90 complexes in the ethylene signaling pathway and define the functional interrelationships between Hsp90 and its co-chaperones within these complexes; 2) determine effects of Hsp90 manipulation by biochemical and genetic means on ERS2, EIN4 and CTR1 protein and complex stability, as well as on ethylene responses at the plant level; and 3) confirm interactions with the newly identified TPR proteins, determine their functions and the functional significance of their interactions with Hsp90.

The proposed research will lead to new functional dimensions of Hsp90, presenting new opportunities for agrobiotechnology.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O 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? O YES X 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)* (Be specific)	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
	Yes X No	Yes X No	Yes X No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 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 X 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		

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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	Yes No			
Rodent	Yes No			
Non-human primate	Yes No			
Other (specify)	Yes 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 X 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Blood (fraction) or other Body Fluid		Yes Unknown		O 1 O 2 2+ O 3
Human Organs or Tissues (unpreserved)		Yes Unknown		O 1 O 2 2+ O 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? X YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X 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
E. coli Strain BL21, DH5 alpha	*Gateway cloning vectors, plant expression vectors, other cloning vectors*	*Bacteria*	*CML10, CBP, CML44, DWF4, TPR and other genes of plant origin*	*Bacteria express proteins when expression vector is used, Plants express the gene and show phenotypes after stress treatments.*

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
YES, complete table below X NO

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved? **The answer to 4.4 to 4.7 is NO**

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

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? 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 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? 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 X NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

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 X No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

7.3 If yes, 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

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES X 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*? _____

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 X 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: _____
O "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:

See E-mail

10.0 Plants

10.1 Do you use plants? X YES ☐ NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _*Arabidopsis thaliana* and *Brassica napus*

10.3 What is the origin of the plant? Canada and USA

10.4 What is the form of the plant (seed, seedling, plant, tree…)? seed, seedling, mature plant

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

10.6 Do you do any modifications to the plant? X YES ☐ NO
If yes, please describe: Overexpression of genes CML10, CBPs, DWF4

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated: _
There is no risk and we dispose off transgenic material after autoclaving

10.8 Is the CFIA permit attached? YES X NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
We have obtained a permit to import seeds of Arabidopsis mutants from resource center and other origins

11.0 Import Requirements

11.1 Will any of the above agents be imported? X YES, please give country of origin_USA ☐ NO
We have obtained a permit to import seeds of Arabidopsis mutants from resource center and other origins

If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES X NO
(not required)

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES X NO
We obtained plant pathogen cultures from within Canada

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE _____

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. X 1 ☐ 2 ☐ 2+ ☐ 3

13.2 Has the facility been certified by OHS for this level of containment?

☐ YES, permit # if on-campus_____

☐ NO, please certify

X NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

All material is disposed by autoclaving

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

Wash thoroughly with soap, wipe with ethanol and apply antibacterial ointment although the material is non-contagious

14.3 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:_ Nov 22, 2010

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

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

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

Date:_____

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

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Biological Agents Registry Form
Date: Mon, 22 Nov 2010 07:30:56 -0500
From: Maryam Khodai-Kalaki <mkhodaik@uwo.ca>
To:priti krishna <pkrishna@uwo.ca>
CC:jstanle2@uwo.ca

E-mail

Dear Priti and Jennifer,

I took Biosafty training course on 14th of October 2009.

Warm regards,
Maryam
----- Original Message -----
From: priti krishna <pkrishna@uwo.ca>
Date: Monday, November 22, 2010 12:11 am
Subject: Biological Agents Registry Form
To: Jennifer Stanley <jstanle2@uwo.ca>
Cc: Maryam Khodai-Kalaki <mkhodaik@uwo.ca>, Rajesh Kumar Gupta <rgupta48@uwo.ca>

> Hi Jennifer
> please find attached. Rajesh is a new student and is still to
> complete his training.
> Maryam will provide her date of course completion.
> thanks for your help
> priti

-------- Original Message --------
Subject: Re: Biological Agents Registry Form: Krishna
Date: Mon, 22 Nov 2010 12:30:13 -0500
From:priti krishna <pkrishna@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>, Ray Williamson <purraw@uwo.ca>

1. I understand that you imported seeds of Arabidopsis mutants from the U.S. (resource center and other origins). Did you have to get a CFIA import permit first and then get the shipment from your American supplier(s)?

Jennifer, we need the permit only if we are using courier and it has to go through customs and safety etc. If we import by regular mail, then we are not asked for the permit although declaration of the material s there.

2. For question 11.3, what plant pathogen cultures do you use?

Leptospharia maculans: DAOM 194244 (Saskatchewan) virulent
Alternaria brassicae: Canadian strain from B. rapa (Saskatchewan)

we just obtained them from

Carolyn Babcock Curator / Conservatrice
Canadian Collection of Fungal Cultures / La collection canadienne de cultures fongiques
Agriculture and Agri-Food Canada/Agriculture et Agroalimentaire Canada
Rm. 1015 K.W. Neatby Bldg./ Salle 1015 l'edifice K.W. Neatby
960 Carling Avenue / 960 avenue CarlingOttawa, Ontario
Canada K1A OC6

babcockc@agr.gc.ca
Telephone/Téléphone: 613-759-1772
Facsimile/Télécopieur: 613-759-1924

E-mail

thanks
priti