

**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	<u>Dr. Mark A. Bernardts</u>
DEPARTMENT	<u>Biology</u>
ADDRESS	<u>NCB 404/B&GS 2025E</u>
PHONE NUMBER	<u>x86477</u>
EMERGENCY PHONE NUMBER(S)	<u>519-200-3414</u>
EMAIL	<u>bernards@uwo.ca</u>

Location of experimental work to be carried out: Building(s) NCB Room(s) 404/464

*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
GRANT TITLE(S): Functional Characterization of Plant Secondary Metabolism/Metabolites

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>
Meg Haggitt	meg.haggitt@uwo.ca	Aug 3, 2011
Jessica Koteles	jkoteles@uwo.ca	Feb 14, 2011
Dimitre Ivanov	divanov2@uwo.ca	Pending (Sept 14 2011 session)
Pooja Sharma	psharm26@uwo.ca	Jan 13, 2011
Anica Bjelica	abjelica@uwo.ca	Cannot confirm date*
Muna Basahi	mbasahi@uwo.ca	Pending
		*Anica is on maternity leave
		until Sept. 2011

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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.

We employ typical microorganisms used in the generation of experimental transgenic plants, the expression of recombinant proteins for biochemical characterization and the generation of "hairy roots" for studying root biology. These include *E. coli* (TOP10, DHalpha), *Agrobacterium tumefaciens*, *Agrobacterium rhizogenes* and yeast (WAT 11). We also use a number of fungal (*Trichoderma hamatum*) and oomycete (*Pythium irregulare*, *Pythium ultimum*, *Phytophthora sojae*) species to study plant-pathogen interactions. Routine culturing, preparation of freezer stocks and standard transformation protocols are used.

We employ a number of vectors/plasmids for this work, including commercially available Gateway vectors, as well as pGEMeasy, pCAMBIA-based vectors for expression analysis. For each of these, we clone-in the gene/promoter constructs that we are interested in studying. We routinely prepare freezer stocks (-80c) of all vectors/plasmids at each stage of development.

All working cultures are grown under standard conditions and disposed of according to UWO policy (i.e., autoclaving with indicator cuultures).

Please include a one page research summary or teaching protocol.

Suberin is a biomacromolecule that plants use to reinforce the cell walls of specific cell types, including root epidermis and endodermis cells. The role of suberin in the resistance of plants to root diseases and tolerance to water stress has caught the interest of breeders and scientists, increasing the need to better understand this under-studied material. The long-term objectives of my research program are to understand the genetic and biochemical control of suberin biosynthesis. Ultimately, I would like to apply this knowledge to enhance suberin deposition and improve plant tolerance to pathogens and drought. In the short term, my objectives are to (i) identify critical genes of suberin biosynthesis, clone them and isolate their promoters, (ii) develop systems in which to manipulate the expression of these genes in tissue relevant to suberin deposition, and (iii) use the systems developed in (ii) to characterize the promoters of these genes, determine critical hormonal and genetic controllers of their expression, and determine their specific role(s) in suberin biosynthesis.

The hydroxylation and further oxidation of the terminal (omega) carbon of fatty acids is a critical step in the biosynthesis of aliphatic suberin monomers, since it introduces a second functional group and allows three-dimensional cross-linking. We have identified and cloned three fatty acid omega hydroxylases (FAWH) from potato and six from soybean. For potato, StFAWH1, has been shown to be tuber specific. For soybean, the expression of the six GmFAWH genes is more complex and we need to confirm which are expressed in roots during normal growth and development. For this we will use a brute force PCR approach involving gene specific primers. The recent (Fall 2010) preliminary release of the *Solanum phureja* genome (<http://potatogenomics.plantbiology.msu.edu/index.html>) has allowed us to clone 1.7 kb of sequence upstream of StFAWH1 and we will now analyse the regulation of expression of this gene (i.e., with GUS and GFP marker genes). Our preliminary data suggests that the first approx. 260 bp 5' of the StFAWH1 transcriptional start site are sufficient for wound-induced GUS expression in transgenic potato hairy roots. We are now poised to explore the regulation of StFAWH1 gene expression in detail. However, this is hampered by the lack of a rapid and robust genetic transformation system in tissues relevant to suberin deposition. While *Arabidopsis* can be rapidly transformed and there are extensive genetic resources available for this species, it does not produce large amounts of suberin. Conversely, potato tubers produce an abundance of suberin; however, obtaining genetically modified potato tubers is laborious and time consuming. To overcome these obstacles, we propose to combine the in vitro propagation of potato microtubers with *Agrobacterium*-mediated genetic transformation to create a system in which genetically modified potato microtuber tissue can be generated on the same time scale as *Arabidopsis* transformants. Similarly, we are adapting a soybean hairy root system, in which *A. rhizogenes*-mediated gene transfer is used to create transgenic soybean roots, to the study of the soybean-*Phytophthora sojae* pathosystem. With this system we will be able to directly test whether altering suberin content of the root affects resistance or susceptibility to *P. sojae*. We will also be able to design experiments to help determine how suberin deposition is regulated in soybean roots, using GmFAWH genes as markers for suberin biosynthesis.

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:

See CFIA Info

1.2 Please complete the table below: (See also, attached table extension, pp 4b)

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
E. coli (TOP10, DHa, BL21)	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1 - 0.5 L		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Saccaromyces cervisiae)	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1 - 0.5 L		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Agrobacterium tumefaciens	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1-0.2 L		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Agrobacterium rhizogenes	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.1-0.2 L		<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 type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input checked="" type="radio"/> Yes <input type="radio"/> No	potato leaves & tubers soybean cotyledons	

The following fungi and oomycetes were obtained from the Canadian Collection of Fungal Cultures. None are genetically modified or used for molecular biological work.

Genus	Species	Isolate No.	Host Species	Location
<i>Trichoderma</i>	<i>hamatum</i>	TH 215090	<i>Cucumis sativus</i>	British Columbia, Canada
<i>Trichoderma</i>	<i>hamatum</i>	TH 215955	<i>Cucumis sativus</i>	British Columbia, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 574	<i>Picea mariana</i>	Ontario, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 901	<i>Cucumis sativus</i>	Alberta, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 486	<i>Phaseolus vulgaris</i>	Netherlands
<i>Pythium</i>	<i>irregulare</i>	BR 779	<i>Triticum aestivum</i>	Transvaal, South Africa
<i>Pythium</i>	<i>irregulare</i>	BR 962	Soil	Wairakei, New Zealand
<i>Pythium</i>	<i>irregulare</i>	BR 598	<i>Panax quinquefolius</i>	Ontario, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 1040	<i>Phaseolus vulgaris</i>	British Columbia, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 1068	<i>Panax quinquefolius</i>	Ontario, Canada
<i>Pythium</i>	<i>irregulare</i>	BR 426	<i>Rhododendron sp.</i>	Alberta, Canada
<i>Pythium</i>	<i>ultimum</i>	BR 638	<i>Pisum sativum</i>	Alberta, Canada

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	<input type="radio"/> Yes <input checked="" type="radio"/> No			
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/UNKNOWN	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
Same as in Table 1.2.	See attached maps			

* 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 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?

- ◆ 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 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 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 _____

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 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 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:

08-09-'11 15:05 FROM-BIOLOGY 1-163 P002/003 F-137

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. potato, soybean, ginseng

10.3 What is the origin of the plant? potato tubers are purchased, soybean seed from Agriculture Canada, ginseng seed and roots from Agriculture Canada

10.4 What is the form of the plant (seed, seedling, plant, tree...)? seeds, tubers, roots

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: potato and soybean tissues are used for molecular biology experimtns; these are generated, used and maintained under sterile, restrictive

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
conditions
The transgenic plants we create to study the role(s) of specific genes in suberin biosynthesis are readily re-generated, as long as we have the appropriate constructs in hand. These materials do not pose any threat to the environment.

10.8 Is the CFIA permit attached? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
These tissues do not require CFIA permits

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ 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

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. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, date of most recent biosafety inspection: _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

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.

N/A

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:

washing well with soapy water & doing accident report. Monitor employee to ensure there are no ill effects.

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: August 3, 2011 _____

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:



E.coli Info



Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are considered to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XL0LR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

----- Original Message -----

Subject:Re: Containment Level Request (agrobacterium LBA4404)
Date:Thu, 05 May 2011 10:46:22 -0400
From:ImportZoopath <ImportZoopath@inspection.gc.ca>
To:Jennifer Stanley <jstanle2@uwo.ca>

Good Morning,

According to our database, **Agrobacterium tumefaciens** would be considered a containment level 1 animal pathogen. If you have further questions, do not hesitate to contact our office.

Thank you,

Steven Burns

Office of Biohazard Containment & Safety, CFIA | Bureau du confinement des biorisques et de la sécurité, ACIA
Government of Canada | Gouvernement du Canada
1400 Merivale, Ottawa ON K1A0Y9
Phone/Tél.: (613) 773-6520
Fax/ Téléc.: (613) 773-6521

ImportZoopath@inspection.gc.ca

----- Original Message -----

Subject:Re: Agrobacterium rhizogenes
Date:Wed, 13 Jul 2011 08:18:26 -0400
From:ImportZoopath <ImportZoopath@inspection.gc.ca>
To:Jennifer Stanley <jstanle2@uwo.ca>

Good Morning,

According to our database, **Agrobacterium rhizogenes** is a level 1 animal pathogen. If you have any further questions, do not hesitate to contact our office.

Thank you,

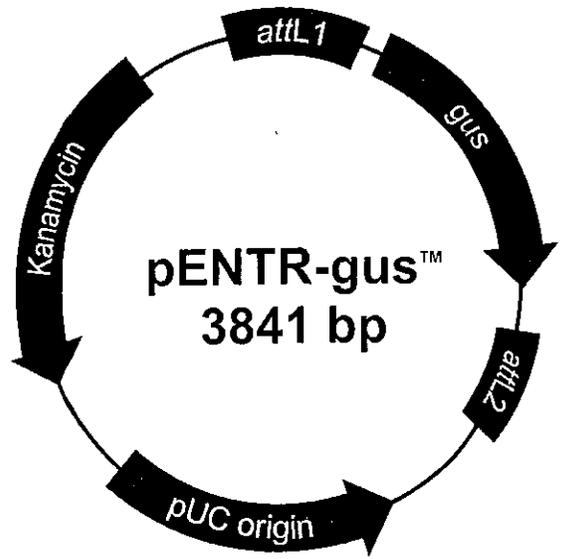
Lauren Baerg

Office of Biohazard Containment & Safety, CFIA | Bureau du confinement des biorisques et de la sécurité, ACIA
Government of Canada | Gouvernement du Canada
1400 Merivale, Ottawa ON K1A0Y9
Phone/Tél.: (613) 773-6520
Fax/ Téléc.: (613) 773-6521

ImportZoopath@inspection.gc.ca

Appendix 1

Vector Maps for vectors used in the Bernards lab.



**Comments for pENTR-gus™
3841 nucleotides**

- attL1: bases 99-198 (complementary strand)
- gus gene: bases 228-2039
- attL2: bases 2041-2140
- pUC origin: bases 2200-2873 (C)
- Kanamycin resistance gene: bases 2990-3805 (C)

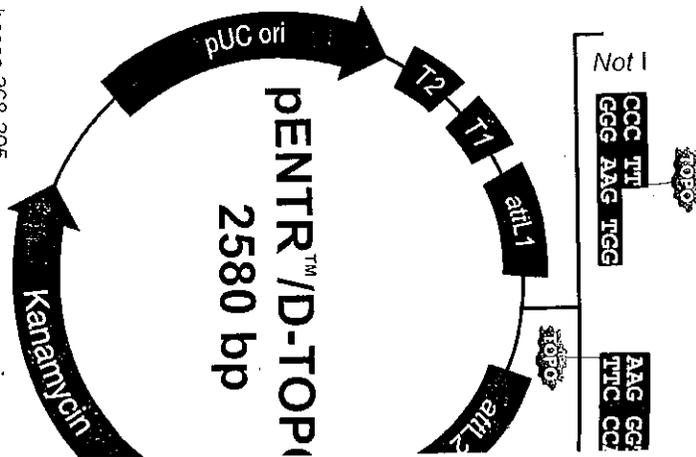
C = complementary strand

Handwritten: Ampicillin 0.15 mg/ml
- culture 0.5 ml

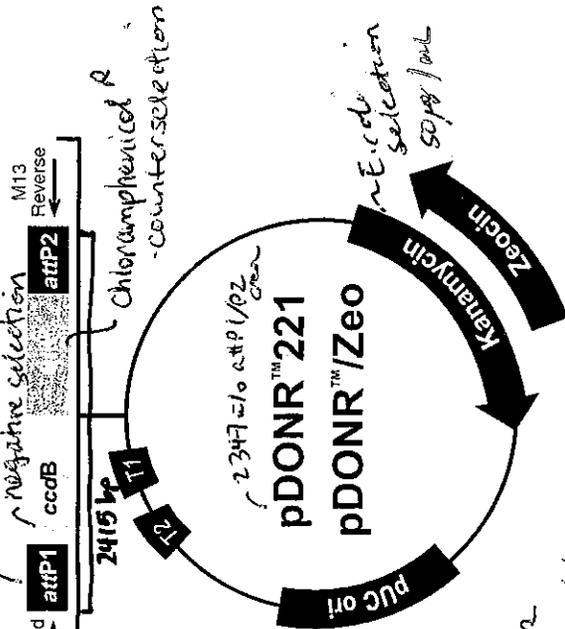
**Comments for pENTR™/D-TOPO®
2580 nucleotides**

- rrnB T2 transcription termination sequence: bases 268-295
- rrnB T1 transcription termination sequence: bases 427-470
- M13 forward (-20) priming site: bases 537-552
- attL1: bases 569-668 (c)
- TOPO® recognition site 1: bases 680-684
- Overhang: bases 685-688
- TOPO® recognition site 2: bases 689-693
- attL2: bases 705-804
- T7 Promoter/priming site: bases 821-840 (c)
- M13 reverse priming site: bases 845-861
- Kanamycin resistance gene: bases 974-1783
- pUC origin: bases 1904-2577

(c) = complementary sequence



Modified from original pDONR201
for improved efficiency
and 1 copy #



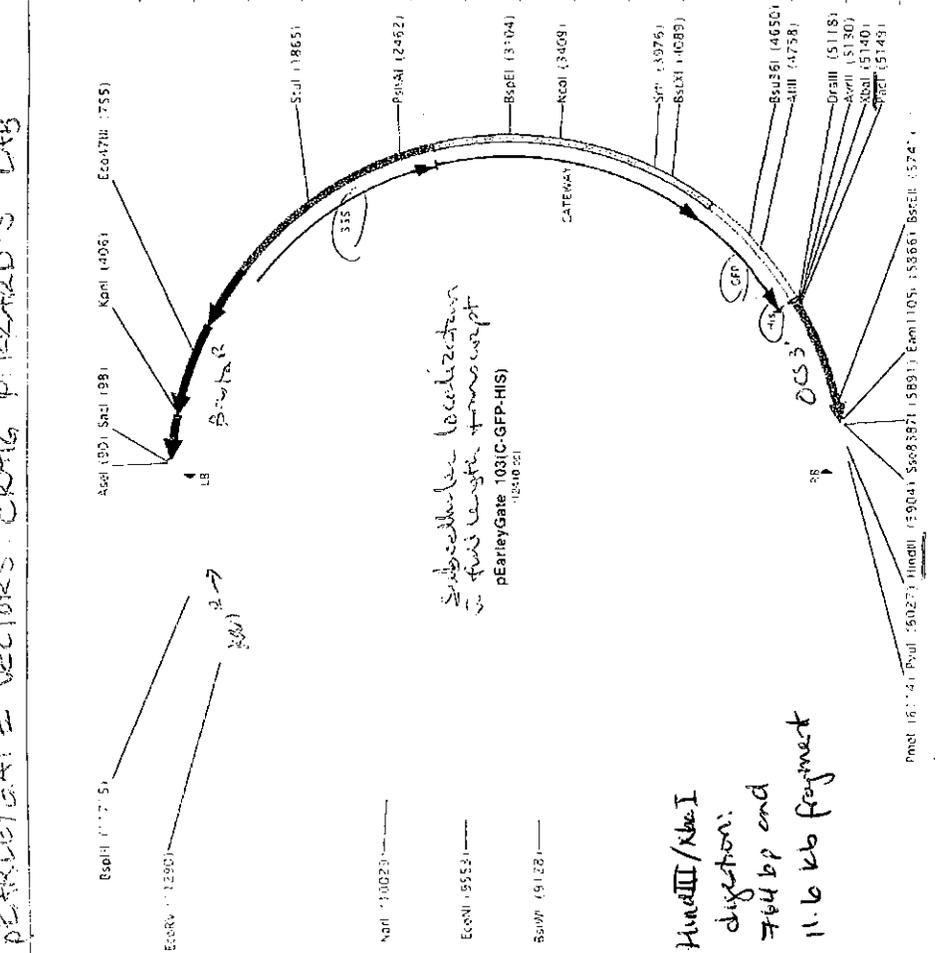
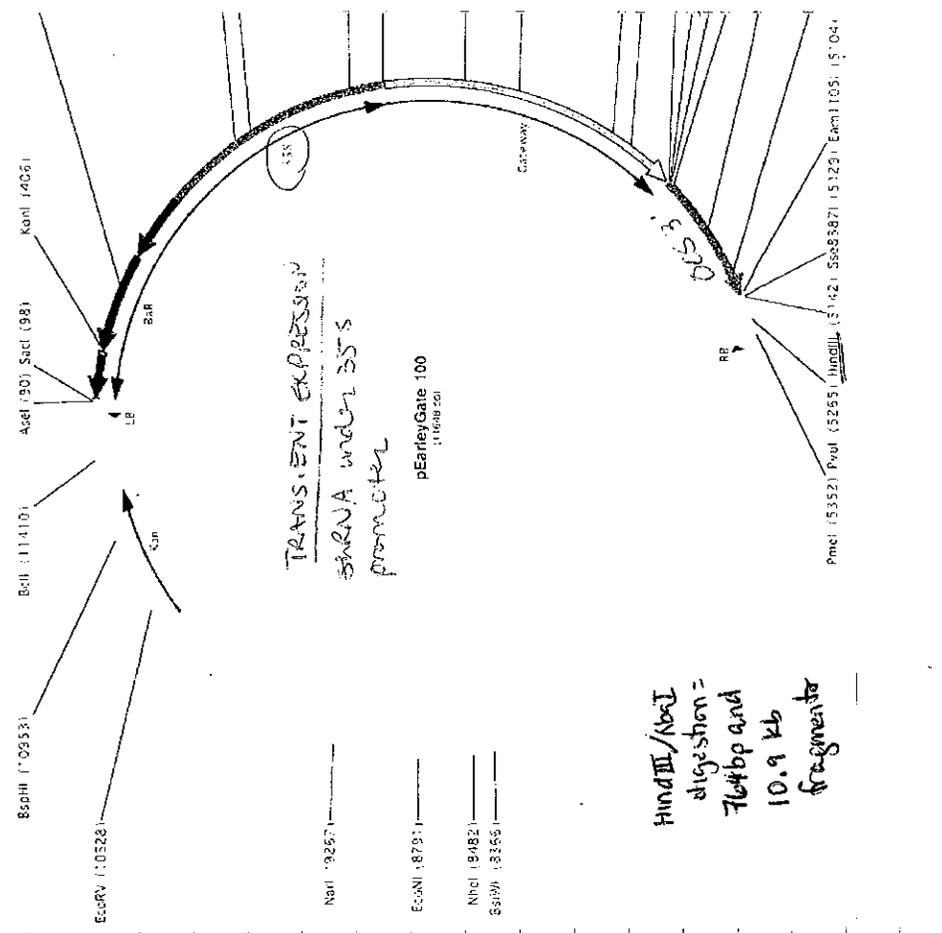
* Entry clone:
DFX between pDONR221
and attB P_{GDH} product

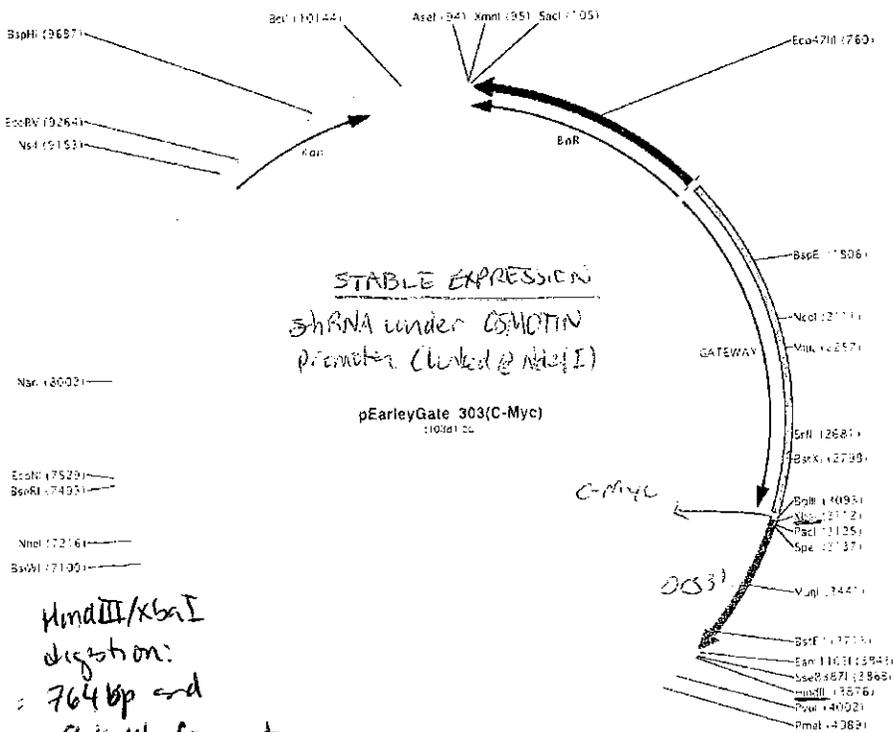
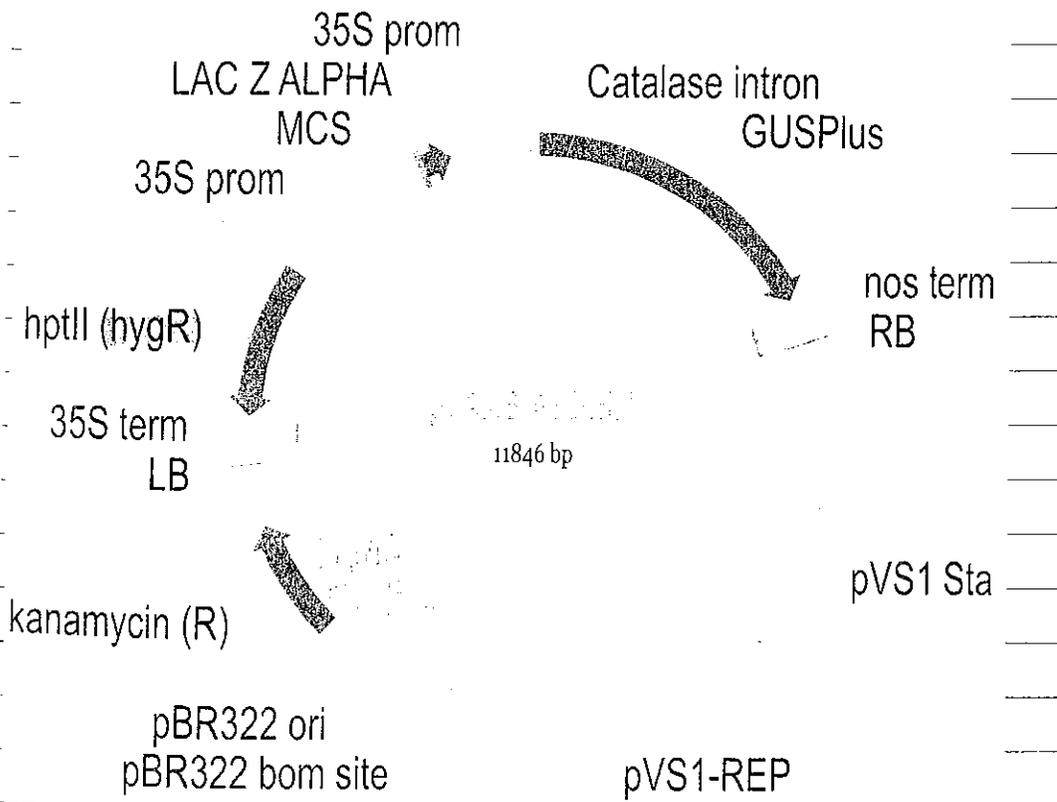
* Expression clone:
LRX between entry
clone and destination
vector (pHGW757.0)

↳ un-recombined pDONR
vectors will not survive b/c

	pDONR™221 4762 nucleotides	pDONR™/Zeo 4291 nucleotides
rrnB T2 transcription termination sequence (c):	268-295	268-295
rrnB T1 transcription termination sequence (c):	427-470	427-470
M13 Forward (-20) priming site:	537-552	537-552
attP1:	570-801	570-801
ccdB gene (c):	1197-1502	1197-1502
Chloramphenicol resistance gene (c): - removed after recombination	1847-2506	1847-2506
attP2 (c):	2754-2985	2754-2985
T7 Promoter/priming site (c):	3000-3019	3003-3022
M13 Reverse priming site:	3027-3043	3027-3043
Kanamycin resistance gene:	3156-3965	---
EM7 promoter (c):	---	---
Zeocin resistance gene (c):	---	3486-3552
PUC origin:	4086-4759	3111-3485
		3615-4288

PEARLEYGATE VECTORS: CRAIG P. KIRKARD'S LAB





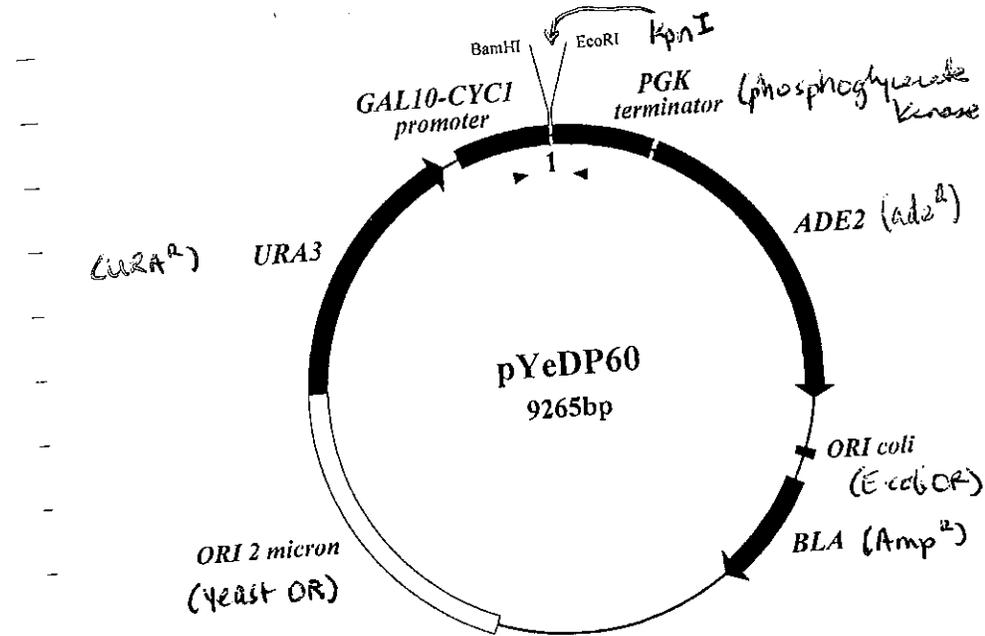


Fig. 1. pYeDP60 map. Arrows indicate the positions of the forward and reverse primers. ADE2 and URA3 represent the yeast genes used for transformation selection. BLA represents the Amp^R gene for E. coli selection. GAL10-CYC1 promoter and PGK terminator represent the promoter and terminator used for cDNA expression of CY ORFs. ORI 2 micron is the replication origin of the yeast 2 μ plasmid, ORI coli represent the replication origin of pUC 19.