

**The University of Western Ontario**  
**BIOLOGICAL AGENTS REGISTRY FORM**  
 Approved Biohazards Subcommittee: October 14, 2011  
 Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://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, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to [jstanle2@uwo.ca](mailto:jstanle2@uwo.ca)) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto: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/](http://www.uwo.ca/humanresources/biosafety/).

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

**If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.**

PRINCIPAL INVESTIGATOR:	<b>Cheryle Seguin</b>
DEPARTMENT:	<b>Physiology and Pharmacology</b>
ADDRESS:	<b>DSB0035B</b>
PHONE NUMBER:	<b>x82977</b>
EMERGENCY PHONE NUMBER(S):	<b>519-474-6239</b>
EMAIL:	<b><a href="mailto:cheryle.seguin@schulich.uwo.ca">cheryle.seguin@schulich.uwo.ca</a></b>

Location of experimental work to be carried out :

Building : <b>Dental Sciences Building</b>	Room(s): <b>0034</b>
Building : <b>Dental Sciences Building</b>	Room(s): <b>0034A</b>
Building : _____	Room(s): _____

**\*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, CIHR

GRANT TITLE(S): **Delineating the Role of the Notochord during Intervertebral Disc Development**  
**Directed differentiation of pancreatic islet precursors from pluripotent human stem cells**  
**Elucidating the Role of CTGF in Intervertebral disc development, health & disease**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): \_\_\_\_\_

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>
Matthew McCann	<a href="mailto:mmccann4@uwo.ca">mmccann4@uwo.ca</a>	11-Jul-2011

Courtney Brooks	cbrook8@uwo.ca	07-Sept-2010
Priya Patel	ppatel86@uwo.ca	23-Jun-2011
Michael Salna	msalna@uwo.ca	07-Mar-2010
Matthew Ernst	mernst2@uwo.ca	28-Sept-2011
Salma Shaikh	sshaik25@uwo.ca	21-Jun-2011
Jacob Bedore	jbedore2@uwo.ca	06-Feb-2012
Michael Sattin	msattin@uwo.ca	4-Oct-2010

**Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.**

All bacteria outlined (DH5A, OneShot OmniMAX chemically competent cells) are used in the project for the purpose of plasmid cloning and production. Transformed bacteria are stored in glycerol stocks in the -80 in a sealed plastic storage box. All cultures of bacteria not used for commercial plasmid prep kits are disposed of after use following bleaching of live cultures. Any solid waste (i.e. tips, plates etc) is autoclaved in labelled biohazard bags before being disposed of.

Human and mouse cell lines used in the research are stored in long term in cryovials in a liquid nitrogen storage tank. Cell lines are cultured in a dedicated culture facility (level 2) and any cells not being used for subsequent experiments are first bleached, then sealed in biohazard bags for autoclaving.

All plasmids used in these experiments are stored either at -20 or 4 C.

**Please include a ONE page research summary or teaching protocol in lay terms.  
Forms with summaries more than one page will not be reviewed.**

The Seguin lab has 2 main areas of research.

**1) Directed differentiation of human embryonic stem cells.**

The long-term goals of this research are to generate clinically relevant organ-specific stem cells from pluripotent stem cell sources (embryonic stem cells (ESC)) with the capacity to direct the repair or regeneration of damaged organs. By understanding and genetically manipulating the pathways that govern cell fate during embryonic development, we will capitalize on the tremendous potential of pluripotent stem cells (ie. their ability to generate all 220 cell types in the body), and effectively direct and limit their developmental potential. We are currently working to apply these strategies to the generation of insulin-secreting pancreatic beta-cells. Previous studies carried out by Dr. Seguin during her postdoctoral fellowship established that expression of SOX17 in hESC was sufficient to restrict stem cell fate and generate endoderm-restricted progenitor cells (the embryonic lineage that will contribute to the liver, lung, pancreas etc.). These cells are the starting point to test the hypothesis that sequential activation of transcription factors in hESC can further restrict cell fate to generate pancreatic progenitor cells. The Seguin lab has generated transgenic hESC that express both SOX17 and NGN3 or PDX1 and is working to determine the phenotype of these cells. These findings may, in the long term, provide a mechanistic approach to generate the unlimited numbers of cells required for beta-cell transplantation from patient-specific pluripotent cells for the >250 million individuals affected worldwide.

**2) Understanding the role of notochord cells during intervertebral disc development and disease.**

Low back pain is the most frequently reported musculoskeletal problem in Canadian adults, often associated with osteoarthritis or rheumatoid arthritis. The most common cause of back pain is disc degeneration, which involves substantial changes to the cellular and extracellular matrix composition of the intervertebral disc (IVD) resulting in impaired physiological function. The Seguin lab has developed unique genetic mouse strains in which CRE recombinase is specifically expressed in notochord-derived cells. We used these strains to conduct fate mapping experiments which established that a subpopulation of cells within the mature IVD (the nucleus pulposus) are derived from the embryonic notochord. Based on this initial characterization, we are conducting studies to determine the phenotype of notochord cells during the process of IVD development and maturation, and assess the contribution of specific molecules to IVD formation using tissue specific knockouts. Using our notochord-specific mice, we have generated lineage-specific reporter mice that enable us to isolate specific IVD cell types based on fluorescent protein expression. Cells isolated from these animals will be maintained in culture to compare specific cell properties between the subpopulations (the nucleus pulposus vs. annulus fibrosus) or to compare wild type and notochord-specific knockout mice.

**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:

	Known a otic ? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
<b>E. coli</b> <i>DH5a competent cells</i>	<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	<b>1 L</b> <b>Invitrogen</b> <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
<i>One Shot® OmniMAX™ 2-T1R Chemically Compete</i>	<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	<b>1L</b> <b>Invitrogen</b> <input checked="" type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
	<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"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

*\*Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:  
[http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA\\_Ecoli\\_list.pdf](http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf)*

Additional Comments: \_\_\_\_\_

## 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="checkbox"/> Yes <input checked="" type="checkbox"/> No		Not applicable
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<b>Mouse intervertebral disc</b>	<b>2009-050</b>
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> 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	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<b>CA1, CA2 human embryonic stem</b>	<b>2</b>	<b>Mount Sinai Hospital, Toronto ON</b>
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<b>mouse embryonic fibroblasts, mESC</b>	<b>2</b>	<b>SickKids Stem Cell Facility, ON</b>
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			

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

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

Additional Comments: **human embryonic stem cells and mouse cells are from academic sources and therefore do not have an associated MSDS**

## 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="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Blood (fraction) or other Body Fluid		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3
Human Organs or Tissues (unpreserved)		<input type="checkbox"/> Yes <input type="checkbox"/> Unknown		<input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 2+ <input type="checkbox"/> 3

Human Organs or Tissues (preserved)		Not Applicable		Not Applicable
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Additional Comments: \_\_\_\_\_

#### 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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
DH5a	pCAGGS pCAGS-ERT2 pCAGS-GRLBD	academic	SOX17 NGN3 PDX1 Brachyury PAX4 GFP RFP	No	No	None - all plasmids are used for differentiation of human embryonic stem cells
One Shot® OmniMAX™ 2-T1R	piggyBac transposon					

**E. coli**

*equivalent if available.*

*the following strains of E. coli:*  
[https://www.cdph.ca/Programs/OPA/Pages/ohs/CFIA\\_Ecoli\\_list.pdf](https://www.cdph.ca/Programs/OPA/Pages/ohs/CFIA_Ecoli_list.pdf)

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.*

4.3.1 Will virus be replication defective?  YES  NO

4.3.2 Will virus be infectious to humans or animals?  YES  NO

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

#### 5.0 Will genetic sequences from the following be involved?

- ◆ HIV  NO  YES, specify
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens  NO  YES, specify

- ◆ SV 40 Large T antigen  NO  YES
- ◆ E1A oncogene  NO  YES
- ◆ Known oncogenes  NO  YES, specify
- ◆ Other human or animal pathogen and or their toxins  NO  YES, specify

5.1 Is any work being conducted with prions or prion sequences?  NO  YES

Additional Comments: \_\_\_\_\_

**6.0 Human Gene Therapy Trials**

6.1 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 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained?  YES, number:  NO  PENDING

**7.0 Animal Experiments**

7.1 Will live animals be used?  YES  NO If NO, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

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

7.6 Will the agent(s) be shed by the animal:  
 YES  NO, please justify:

**8.0 Use of Animal species with Zoonotic Hazards**

8.1 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 9.0

8.2 Will live animals be used?  YES  NO

8.3 If YES, please specify the animal(s) used:

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

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

## 9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used?  YES  NO If **NO**, please proceed to Section 10.0

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

9.3 What is the LD<sub>50</sub> (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time\*?

9.5 How much of the toxin or hormone is stored\*?

9.6 Will any biological toxins or hormones be used in live animals?  YES  NO  
If **YES**, Please provide details:

\*For information on biosecurity requirements, please see:  
[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

Additional Comments: \_\_\_\_\_

## 10.0 Insects

10.1 Do you use insects?  YES  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 insect?

10.4 What is the life stage of the insect?

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

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

10.7 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:

## 11.0 Plants

- 11.1 Do you use plants?  YES  NO - If **NO**, please proceed to Section 12.0
- 11.2 If YES, please give the name of the species.
- 11.3 What is the origin of the plant?
- 11.4 What is the form of the plant (seed, seedling, plant, tree...)?
- 11.5 What is your intention?  Grow and maintain a crop  "One-time" use
- 11.6 Do you do any modifications to the plant?  YES  NO  
If yes, please describe:
- 11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
- 11.8 Is the CFIA permit attached?  YES  NO  
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

## 12.0 Import Requirements

- 12.1 Will any of the above agents be imported?  YES, country of origin  NO  
If **NO**, please proceed to Section 13.0
- 12.2 Has an Import Permit been obtained from HC for human pathogens?  YES  NO
- 12.3 Has an import permit been obtained from CFIA for animal or plant pathogens?  YES  NO
- 12.4 Has the import permit been sent to OHS?  YES, please provide permit #  NO

## 13.0 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.

**An X in the check box indicates you agree with the above statement...**   
**Enter Your Name** Cheryle Seguin **Date:** Jan 31, 2012

**14.0 Containment Levels**

14.1 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

14.2 Has the facility been certified by OHS for this level of containment?  
 YES, location and date of most recent biosafety inspection: **9/1/2011**  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-0236**

**15.0 Procedures to be Followed**

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents?  YES  NO  
If **YES** please describe:

15.2 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:  
**Exposure to biological agents in the form of accidental splash will be dealt with by flushing the exposed area with water, using the eye wash station, or sink as applicable. Needles are not used with the agents in my laboratory.**

15.3 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.shs.uwo.ca/workplace/workplacehealth.html>

**An X in the check box indicates you agree with the above statement...**   
**Enter Your Name Cheryle Seguin Date: Jan 31, 2012**

15.4 Additional Comments: \_\_\_\_\_

**16.0 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:



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 20<sup>th</sup>, 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

<b>1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING</b>
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**Product code** 500516  
**Product name** OmniMax One Shot V

**Company/Undertaking Identification**

INVITROGEN CORPORATON  
 5791 VAN ALLEN WAY  
 PO BOX 6482  
 CARLSBAD, CA 92008  
 760-603-7200

INVITROGEN CORPORATION  
 2270 INDUSTRIAL STREET  
 BURLINGTON, ONT  
 CANADA L7P 1A1  
 800-263-6236

GIBCO PRODUCTS  
 INVITROGEN CORPORATION  
 3175 STALEY ROAD P.O. BOX 68  
 GRAND ISLAND, NY 14072  
 716-774-6700

<b>2. COMPOSITION/INFORMATION ON INGREDIENTS</b>
--

**Hazardous/Non-hazardous Components**

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	7-13

The product contains no substances which at their given concentration, are considered to be hazardous to health

<b>3. HAZARDS IDENTIFICATION</b>
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**Emergency Overview**

Components of the product may be absorbed into the body through the skin

**Form**  
 Liquid

### 3. HAZARDS IDENTIFICATION

#### Principle Routes of Exposure/

#### Potential Health effects

**Eyes** Mild eye irritation.  
**Skin** moderate skin irritation. Components of the product may be absorbed into the body through the skin.  
**Inhalation** No information available  
**Ingestion** May be harmful if swallowed.

#### Specific effects

**Carcinogenic effects** No information available  
**Mutagenic effects** No information available  
**Reproductive toxicity** No information available  
**Sensitization** No information available

#### Target Organ Effects

No information available

#### HMIS

Health	1
Flammability	0
Reactivity	0

### 4. FIRST AID MEASURES

**Skin contact** Wash off immediately with plenty of water  
**Eye contact** Rinse thoroughly with plenty of water, also under the eyelids.  
**Ingestion** Never give anything by mouth to an unconscious person  
**Inhalation** Move to fresh air  
**Notes to physician** Treat symptomatically.

### 5. FIRE-FIGHTING MEASURES

**Suitable extinguishing media** Dry chemical  
**Special protective equipment for firefighters** Wear self-contained breathing apparatus and protective suit

### 6. ACCIDENTAL RELEASE MEASURES

**Personal precautions** Use personal protective equipment  
**Methods for cleaning up** Soak up with inert absorbent material.

### 7. HANDLING AND STORAGE

**Handling** No special handling advice required  
**Storage** Keep in properly labelled containers

### 8. EXPOSURE CONTROLS / PERSONAL PROTECTION

#### Occupational exposure controls

#### Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

**Engineering measures** Ensure adequate ventilation, especially in confined areas

**Personal protective equipment**

**Respiratory protection** In case of insufficient ventilation wear suitable respiratory equipment  
**Hand protection** Impervious butyl rubber gloves. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes.. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes.

**Eye protection** Safety glasses with side-shields  
**Skin and body protection** Lightweight protective clothing.  
**Hygiene measures** Handle in accordance with good industrial hygiene and safety practice  
**Environmental exposure controls** Prevent product from entering drains.

**9. PHYSICAL AND CHEMICAL PROPERTIES**

**General Information**

**Form** Liquid

**Important Health Safety and Environmental Information**

**Boiling point/range** °C No data available °F No data available  
**Melting point/range** °C No data available °F No data available  
**Flash point** °C No data available °F No data available  
**Autoignition temperature** °C No data available °F No data available  
**Oxidizing properties** No information available  
**Water solubility** soluble

**10. STABILITY AND REACTIVITY**

**Stability** Stable.  
**Materials to avoid** No information available  
**Hazardous decomposition products** No information available  
**Polymerization** Hazardous polymerisation does not occur.

**11. TOXICOLOGICAL INFORMATION**

**Acute toxicity**

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

**Principle Routes of Exposure/**

**Potential Health effects**

**Eyes** Mild eye irritation.  
**Skin** moderate skin irritation. Components of the product may be absorbed into the body through the skin.  
**Inhalation** No information available  
**Ingestion** May be harmful if swallowed.

**Specific effects**

**Carcinogenic effects** No information available  
**Mutagenic effects** No information available  
**Reproductive toxicity** No information available

Sensitization No information available

Target Organ Effects No information available

## 12. ECOLOGICAL INFORMATION

Ecotoxicity effects No information available.  
Mobility No information available.  
Biodegradation Inherently biodegradable.  
Bioaccumulation Does not bioaccumulate.

## 13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

## 14. TRANSPORT INFORMATION

### IATA

Proper shipping name Not classified as dangerous in the meaning of transport regulations  
Hazard Class No information available  
Subsidiary Class No information available  
Packing group No information available  
UN-No No information available

## 15. REGULATORY INFORMATION

### International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

### U.S. Federal Regulations

#### SARA 313

This product is not regulated by SARA.

#### Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)

This product does not contain HAPs.

### U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

### California Proposition 65

This product does not contain chemicals listed under Proposition 65

### WHMIS hazard class:

D2B Toxic materials  
B3 Combustible liquid



This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

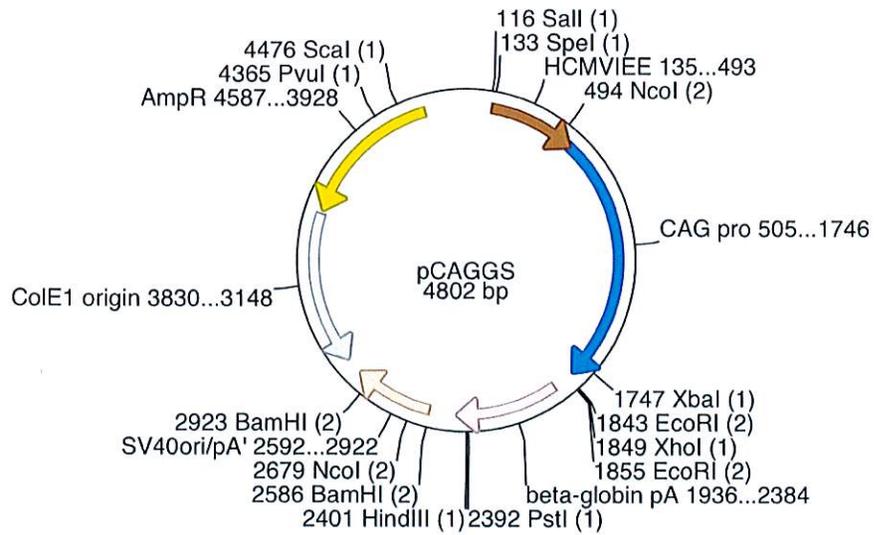
## 16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

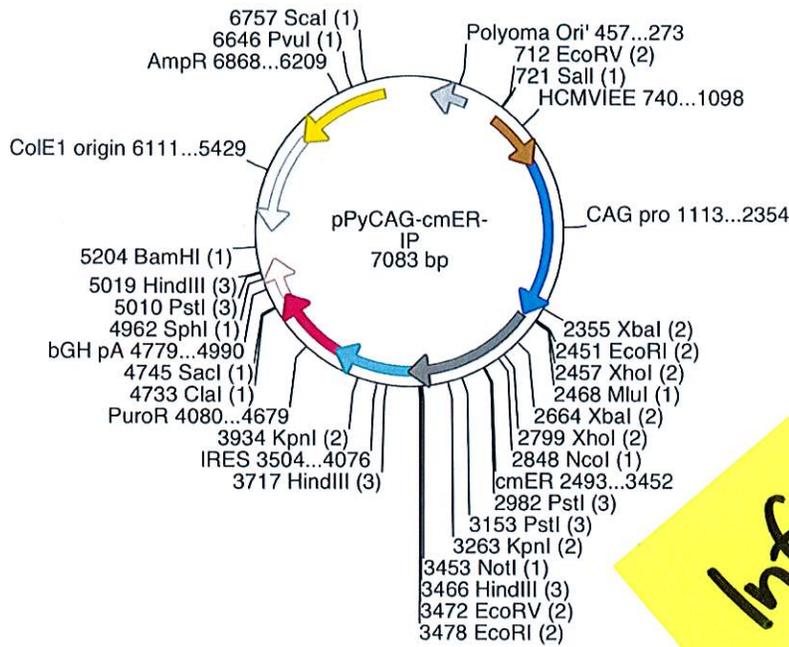
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**End of Safety Data Sheet**

## pCAGGS

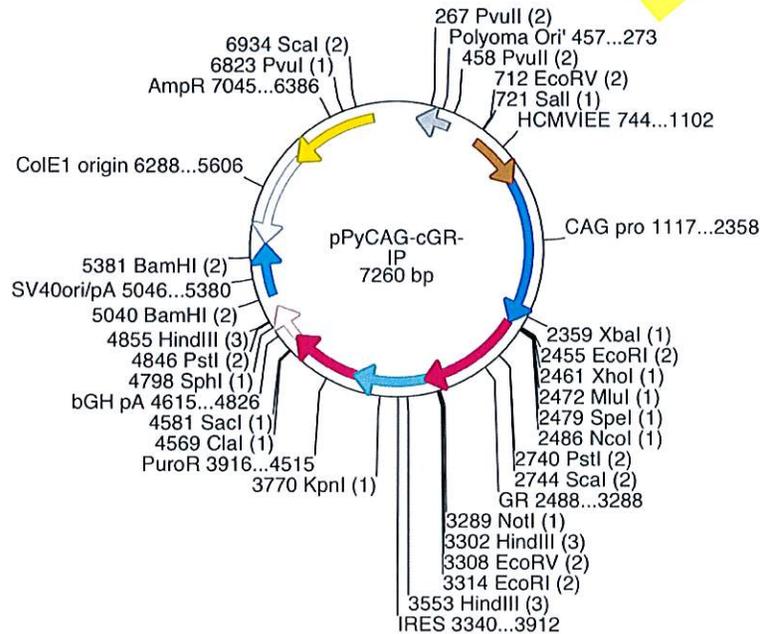


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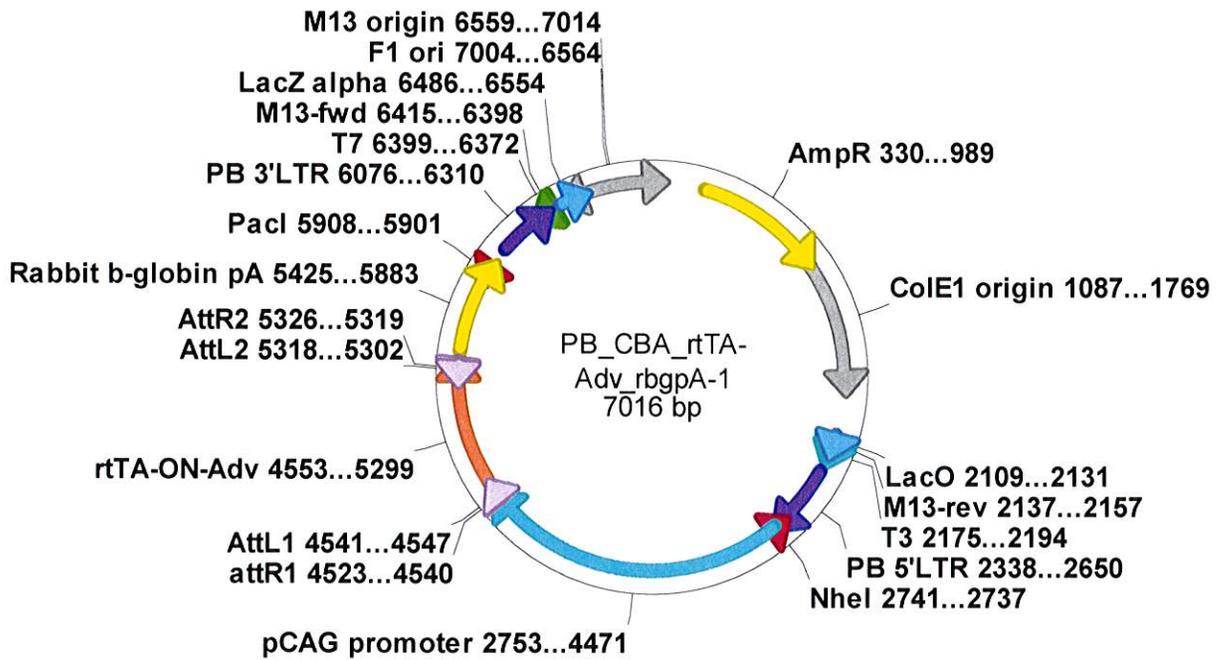


*Info on Plasmids*

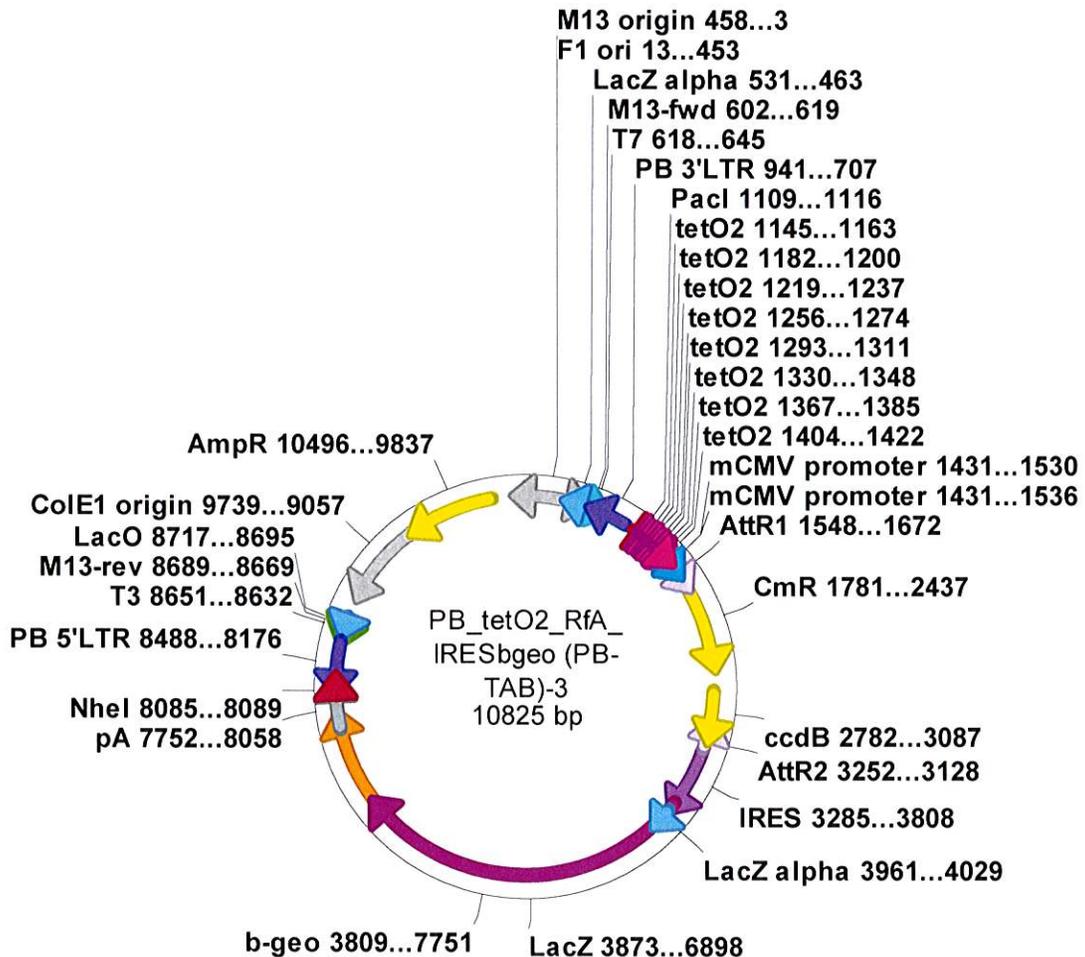
## pCAGGS-GRLBD



**piggyBac Transposase plasmids: 1) rtTA vector**



**piggyBac Transposase plasmids: 2) TetO expression vector**



**piggyBac Transposase plasmids: 3) transposase vector**

Name of plasmid: pc2043 Date: Nov. 3, 2001  
 Bacteria stock location: \_\_\_\_\_ Plasmid DNA location: \_\_\_\_\_  
 Constructed by: Chengyi Lu Vector: Not-CAG-puro  
 Antibiotic resistance: amp Bacterial strain: DH5 $\alpha$   
 Plasmid description: To amplify fragment with primer # 1378 & # 1359 from cdk-10  
to cut the amplified PCR product with BamHI - NotI  
to cut the Not-CAG-puro vector with BamHI - NotI and remove the backbone  
to ligate  
 Special comments: Not-CAG-puro-~~cdk-10~~-pBAC-K10 (transposase)

