

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
Approved Biohazards Subcommittee: October 14, 2010  
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).

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](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/)

PRINCIPAL INVESTIGATOR Wing-Yiu Choy  
 DEPARTMENT Biochemistry  
 ADDRESS Medical Sciences Building Room 302  
 PHONE NUMBER x83161  
 EMERGENCY PHONE NUMBER(S) 519-697-3888  
 EMAIL jchoy4@uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 314, 314B

\*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: CIHR  
 GRANT TITLE(S): Structural studies of intrinsically disordered proteins

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>
Anne Brickenden	<a href="mailto:abricken@uwo.ca">abricken@uwo.ca</a>	09-May-2008
Elio Cino	<a href="mailto:ecino@uwo.ca">ecino@uwo.ca</a>	02-Oct-2008
Halema Khan	<a href="mailto:hk2010@uwo.ca">hk2010@uwo.ca</a>	02-Oct-2008
Andrzej Maciejewski	<a href="mailto:amaciej@uwo.ca">amaciej@uwo.ca</a>	24-May-2009
Sarah Skinner	<a href="mailto:sskinn@uwo.ca">sskinn@uwo.ca</a>	21-Sept-2010
Jennifer Brock	<a href="mailto:jbrock26@uwo.ca">jbrock26@uwo.ca</a>	27-Sept-2010

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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.**

Bacteria: *e.coli* for recombinant protein production.

Stored @ 4°C, -20°C, -80°C.

Growth in incubators in M314, M314B.

Liquid waste and growth vessels treated with Wescodyne® bactericidal detergent before flushing to drain.

Solid waste treated by autoclaving before garbage disposal.

cPrP protein:

Stored @ 4°C, -20°C, in M314 and -80°C in M365.

Liquid waste is Bleach treated before flushing to drain.

Solid waste and glassware is Bleach treated before flushing to drain or autoclaving and garbage disposal.

**Please include a one page research summary or teaching protocol.**

The focus of this project is on the structural characterization, and dynamic studies of IDPs. Due to their structural plasticity, and the ability to interact with multiple targets, IDPs are frequently found to be involved in signal transduction and transcription regulation. The goal of our research is to understand the molecular mechanisms by which IDPs interact with targets, and carry out their functions. Currently, we are investigating the structure-function relationships of several disease-associated IDPs. TC-1, and Chibby are two IDPs involved in the Wnt signaling pathway. NMR, and other biophysical techniques are used to dissect the molecular mechanism by which TC-1 interacts with Cby, and to establish the mechanism used by TC-1 and  $\beta$ -catenin for competitive binding to Cby. The result will provide insights into the regulation of cancer-associated genes in the Wnt/ $\beta$ -catenin signaling pathway.

Another IDP that we are investigating is ProT $\alpha$ . It is a small, acidic protein with multiple functions. It plays an essential role in cell proliferation, apoptosis, and is involved in transcriptional regulation of oxidative stress-protecting genes. Structural characterization of ProT $\alpha$  demonstrates that this protein is largely unstructured under physiological conditions but undergoes partial folding upon binding to zinc. We have proposed that this metal-binding property acts as an "entropic switch" for its interaction with the protein target Keap1 in the oxidative stress response. Extensive structural, and dynamic studies are carried out in our lab to elucidate the mode of binding between ProT $\alpha$  and Keap1, and the mechanism by which zinc mediates the interaction of these two proteins.

We are also interested in the macromolecular crowding effects on the stability and dynamics of proteins, with the focus particularly on IDPs. Effects of molecular crowding on protein folding, and stability are usually studied by conventional spectroscopic techniques such as circular dichroism, or fluorescence spectroscopy. Even though these methods can be used to detect global structural changes of proteins under different environments, detailed structural and dynamic information at atomic level cannot be obtained. By including multiple disordered proteins with unique structural characteristics in this study, more general effects of molecular crowding on protein disordered state ensembles can be derived. Crowding agents with different structural properties are used to extensively investigate the crowding effects on the conformational propensities and dynamics of these proteins.

## 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:

\_\_\_\_\_  
 \_\_\_\_\_

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
<u>e.coli</u>	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	Yes <input checked="" type="checkbox"/> No	4L	Novagen (Merck)	<input checked="" type="checkbox"/> 1 2 2+ 3
	Yes	Yes	Yes			1 2 2+ 3
E. coli BL21 (DE3), DHS- alpha, Rosetta						1 2 2+ 3
	NO	NO	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  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 type(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		Yes Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		Yes Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		Yes 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
See attachments				

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

\*\* Please attach a plasmid map.

↑  
E. coli BL21 (DE3),  
DH5-alpha, Rosetta

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:  
 \_\_\_\_\_  
 \_\_\_\_\_



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. \_\_\_\_\_

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? Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? YES 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? YES NO  
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:  
\_\_\_\_\_  
\_\_\_\_\_

**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

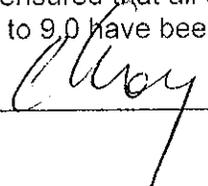
11.4 Has the import permit been sent to OHS? YES, please provide permit # \_\_\_\_\_ NO

**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.

⇒ Bleach treatment of cPrP Protein and vessels is considered beyond the requirement.

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:

⇒ Agent exposure would be treated by topical decontamination with bactericidal soap, followed by flushing with tap water. Bench surfaces used for bacterial work are protected by bench paper and are changed weekly. Small spills on other surfaces are dealt with immediately treating with Wescodyne® or 70% Isopropanol in water. Larger spills are dealt with a lab "Spill kit" containing gloves, spill pillows, autoclave bags and 175mL of Roccal disinfectant.

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 \_\_\_\_\_ *Choy* \_\_\_\_\_ Date: Feb 23, 2011

**15.0 Approvals**

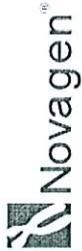
1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

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:



Customer Number

**1. Identification of the substance/preparation and of the company/undertaking**

Product name: BL21(DE3) Glycerol Stock  
 Catalogue number: 69387  
 Supplier: Manufactured by EMV Biosciences Inc.  
 4410  
 Madison, WI 53719  
 (608)235-6110  
 (608)237-0144  
 FAX: (608)235-1388  
 P.O. Box 13087  
 La Jolla, CA 92039-2087  
 (608)450-5598  
 (608)454-3417  
 FAX: (608)453-3552  
 Not available  
 Supplier telephone number: Call ChemService  
 (800)424-3081 (in the U.S.A.)  
 (314)527-2888 (in the U.S.A.)

**2. Composition / information on ingredients**

Substance/Preparation	Substance
Chemical name* BL21(DE3) Glycerol Stock	EC Number Not available CAS No. R-Phrases

**3. Hazards identification**

Physical hazard: Not applicable  
 Health hazard: No specific hazard

**4. First-aid measures**

**Inhalation:** If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.  
**Ingestion:** Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If this material has been swallowed, call a physician immediately. Loosen tight clothing such as a collar, tie, belt or waistband.  
**Skin Contact:** In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.  
**Eye Contact:** Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.  
 Aspiration or prolonged exposure is not known to aggravate medical condition.

**5. Fire-fighting measures**

Flammability of the Product: May be combustible at high temperature  
 Extinguishing Media: Suitable  
 SMALL FIRE: Use DRY chemical powder  
 LARGE FIRE: Use water spray, fog or foam. Do not use water jet  
 Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear  
 Precaution for fire-fighters: Be sure to use an approved/certified respirator or equivalent

**6. Accidental release measures**

Personal precautions: Splash goggles, Full suit, Boots, Gloves. Suggested protective clothing might not be sufficient, consult a specialist BEFORE handling this product.  
 Small Spill and Leak: Absorb with an inert material and put the spilled material in an appropriate waste disposal.  
 Large Spill and Leak: Absorb with an inert material and put the spilled material in an appropriate waste disposal.

**7. Handling and storage**

Handling: Keep away from heat. Keep away from sources of ignition. Empty containers pose a fire risk, evaporate the residue under a fume hood. Ground all equipment containing material. Do not breathe gas/fumes/vapor/spray.  
 Storage: Keep container tightly closed. Keep container in a cool, well-ventilated area. Do not store above 70°C (164°F).  
 Use original container.

**8. Exposure controls/personal protection**

Engineering measures: Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapors below their respective threshold limit value. Ensure that rewash stations and safety showers are proximal to the contamination location.  
 Hygiene measures: Wash hands after handling compounds and before eating, smoking, using lavatory, and at the end of any work shift.  
 Occupational Exposure Limits: Not available

**Personal protective equipment**

- Skin and hand: Lab coat
- Eyes: Safety glasses
- Protective Clothing (PPE):

**9. Physical and chemical properties**

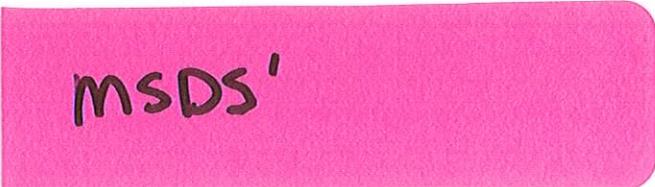
- Physical state: Liquid
- Color: Not available
- Molecular Weight: Not available
- Solubility: Not available
- Flash point: Not available
- Explosive properties: Risks of explosion of the product in presence of mechanical impact. Not available. Risks of explosion of the product in presence of static discharge. Not available.

**10. Stability and reactivity**

Stability: The product is stable  
 Conditions to avoid: Not available

**11. Toxicological information**

RT50/50: Not available  
 Local effects: Not available  
 Skin irritation: Not available  
 Acute toxicity: LD50 Not available, LC50 Not available  
 Chronic toxicity: Repeated or prolonged exposure is not known to aggravate medical condition  
 Other Toxic Effects on Humans: Not available  
 No specific information is available in our database regarding the other toxic effects of this material for human health.  
 To the best of our knowledge, the toxicological properties of this product have not been thoroughly investigated.  
 Carcinogenic effects: Not available



Metabolic effects: Not available  
 Reproductive effects: Not available  
 Teratogenic effects: Not available

## 12. Ecological information

Ecotoxicity: Not available  
 Toxicity to fish: Not available  
 Toxicity to birds: Not available

## 13. Disposal considerations

Waste must be disposed of in accordance with federal, state and local environmental control regulations. Contaminated packaging:

## 14. Transport information

Transportation Name: Not available

UN Number: Not available

ADR: Not available

## 15. Regulatory information

REACH: Not available

Risk Phrases: Not available

REACH: Not available

The product is not classified according to GHS (Hazard Symbols)

7502A: No products were found

SARA 302/304: No products were found

SARA 303: No products were found

SARA 311: No products were found

SARA 312: No products were found

SARA 313: No products were found

SARA 314: No products were found

SARA 315: No products were found

SARA 316: No products were found

SARA 317: No products were found

SARA 318: No products were found

SARA 319: No products were found

SARA 320: No products were found

SARA 321: No products were found

SARA 322: No products were found

SARA 323: No products were found

SARA 324: No products were found

SARA 325: No products were found

SARA 326: No products were found

SARA 327: No products were found

SARA 328: No products were found

SARA 329: No products were found

SARA 330: No products were found

SARA 331: No products were found

SARA 332: No products were found

SARA 333: No products were found

SARA 334: No products were found

SARA 335: No products were found

SARA 336: No products were found

SARA 337: No products were found

SARA 338: No products were found

SARA 339: No products were found

SARA 340: No products were found

SARA 341: No products were found

SARA 342: No products were found

SARA 343: No products were found

SARA 344: No products were found

SARA 345: No products were found

SARA 346: No products were found

SARA 347: No products were found

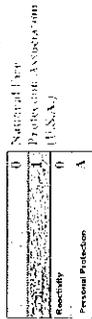
SARA 348: No products were found

SARA 349: No products were found

SARA 350: No products were found

## 16. Other information

Information Material:  
 Information System:  
 U.S.A.:



Material Name:

To the best of our knowledge, the information contained herein is accurate. However, neither the above named supplier nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards, and should be used with caution. Although certain hazards are described herein, we cannot guarantee that there are no other hazards that exist. This product is intended for research use only.

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

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,

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

**Subject:** Re: Biological Agents Registry Form (Choy)

**From:** James Choy <jchoy4@uwo.ca>

**Date:** Tue, 29 Mar 2011 15:18:48 -0400

**To:** Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Sorry that I missed your call. The E. coli strains that we use are BL21, DH5-alpha, and Rosetta (they are all E. coli).

Regards,

James Choy

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

**Subject:**Re: Containment Level Request

**Date:**Tue, 29 Mar 2011 16:10:51 -0400

**From:**Permit-Permis <permitpermis@phac-aspc.gc.ca>

**To:**Jennifer Stanley <jstanle2@uwo.ca>

Dear Jennifer Stanley,

Our classification for **E. coli Rosetta** (strain B) is Risk Group 1.

However, is you intent to introduce a gene coding for a toxin, the recombinant bacteria would be consider as Risk Group 2.

Best

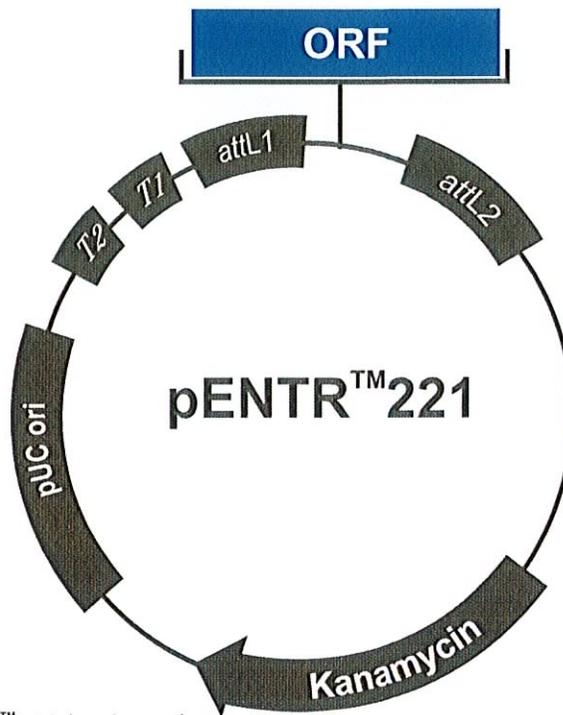
Normand Labbé

Pathogen Regulation Directorate / Direction de la réglementation des agents  
pathogènes  
Public Health Agency of Canada/ Agence de santé publique du Canada  
100 ch. Colonnade Rd. AL: 6201A Ottawa, Ontario, Canada, K1A 0K9  
Tel: (613) 957-1779/ Fax: (613)941-0596  
<http://www.phac-aspc.gc.ca/lab-bio/index-eng.php>

The Pathogen Regulation Directorate turnaround time from receipt of a complete application or checklist to permit or compliance letter is on the order of 20 business days.

PLASMID NAME	SOURCE OF PLASMID	GENE	ON TRANSFORMATION
pcDNA3 Apaf-1	G Nunez, Ann Arbor USA	Human Apaf-1	Ampicillin Resistance
pDEST 14	J. Forman-Kay, Toronto		Ampicillin Resistance
pDEST 15 Neh2	Choy Lab Construction	Human Neh2	Ampicillin Resistance
pDEST 15 TC-1	Choy Lab Construction	Human TC-1	Ampicillin Resistance
pDEST 17	J. Forman-Kay, Toronto		Ampicillin Resistance
pDEST 17 mKelch	Choy Lab Construction	Mouse Kelch	Ampicillin Resistance
pDEST 17 Neh2	Choy Lab Construction	Human Neh2	Ampicillin Resistance
pDEST 17 Nrf2	Choy Lab Construction	Human Nrf2	Ampicillin Resistance
pDEST 17 P8	Choy Lab Construction	Human P8	Ampicillin Resistance
pDEST 17 TPR1	Choy Lab Construction	Mouse TPR1 domain of STI-1	Ampicillin Resistance
pDEST 17 TPR2a	Choy Lab Construction	Mouse TPR2a domain of STI-1	Ampicillin Resistance
pDEST 17 TPR2a delta	Choy Lab Construction	Mouse TPR2a delta	Ampicillin Resistance
pDEST 17 14.3.3	Choy Lab Construction	Human 14.3.3	Ampicillin Resistance
pDEST 17 b-Catenin	Choy Lab Construction	Human b-Catenin	Ampicillin Resistance
pDEST 17 Chibby	Choy Lab Construction	Human Chibby	Ampicillin Resistance
pDEST 24	J. Forman-Kay, Toronto		Ampicillin Resistance
pDEST 42	J. Forman-Kay, Toronto		Ampicillin Resistance
pDEST His MBP	Addgene		Ampicillin Resistance
pDEST His MBP N-term Chibby	Choy Lab Construction	Human Chibby	Ampicillin Resistance
pDEST His MBP TC-1	Choy Lab Construction	Human TC-1	Ampicillin Resistance
pDONR 201	J. Forman-Kay, Toronto		Kanamycin Resistance
pENTR	J. Forman-Kay, Toronto		Kanamycin Resistance
pENTR 221 14.3.3	GeneCopoeia	Human 14.3.3	Kanamycin Resistance
pENTR 221 b-Catenin	Invitrogen	Human b-Catenin	Kanamycin Resistance
pENTR 221 Nrf2	Invitrogen	Human NRF2	Kanamycin Resistance
pET 11a	Novagen		Ampicillin Resistance
pET 11a TC-1		Human TC-1	Ampicillin Resistance
pET 15b hKelch	C. Eberle, MO USA	Human Kelch	Ampicillin Resistance
pET 15b TC-1	M. Sunde, U of Sydney, Australia	Human TC-1	Ampicillin Resistance
pET 17b ING	Y. Bai, NIH USA	Human ING	Ampicillin Resistance
pGEX 4T	B. Sanwal Lab, Canada		Ampicillin Resistance
pGEX 4T AANAT	J. Boutin, France	Sheep N-acetyltransferase	Ampicillin Resistance
pGEX 4T1 b-Cat 3	Choy Lab Construction		Ampicillin Resistance
pGEX 4T-1 TEV	S. Li Lab, Canada	TEV Protease	Ampicillin Resistance
pGEX 4T1 TEV Chibby	Choy Lab Construction	Human Chibby	Ampicillin Resistance
pHP12a Prothymosin Alpha	A. Vartapetian, Moscow Russia	Human Prothymosin Alpha	Ampicillin Resistance
pReceiver B03y P8	GeneCopoeia	Human P8	Ampicillin Resistance
pRK793 His TEV	G. Shaw Lab, Canada	TEV Protease	Ampicillin Resistance
pRSETA mPrP 23-231	K. Wuthrich, Switzerland	Mouse cPrP	Ampicillin Resistance
pRSETA mPrP 90-232	K. Wuthrich, Switzerland	Mouse cPrP	Ampicillin Resistance
pT7-7 alpha-Synuclein	M Volles, Harvard USA	Human alpha-Synuclein	Ampicillin Resistance
pTrc His STI-1	Prado Lab, Canada	Mouse STI-1	Ampicillin Resistance
pTrc His STI-1 delta	Prado Lab, Canada	Mouse STI-1 delta	Ampicillin Resistance
pYX-Asc mKelch	ATCC, USA	Mouse Kelch	Ampicillin Resistance

## Section 4



**Comments for pENTR™221 (no insert)  
2546 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 569-667 (complementary strand)

ORF insertion site: bases 668-669

*attL2*: bases 671-770

M13 reverse priming site: bases 811-827

Kanamycin resistance gene: bases 940-1749

pUC origin: bases 1870-2543



## pET-11a-d Vectors

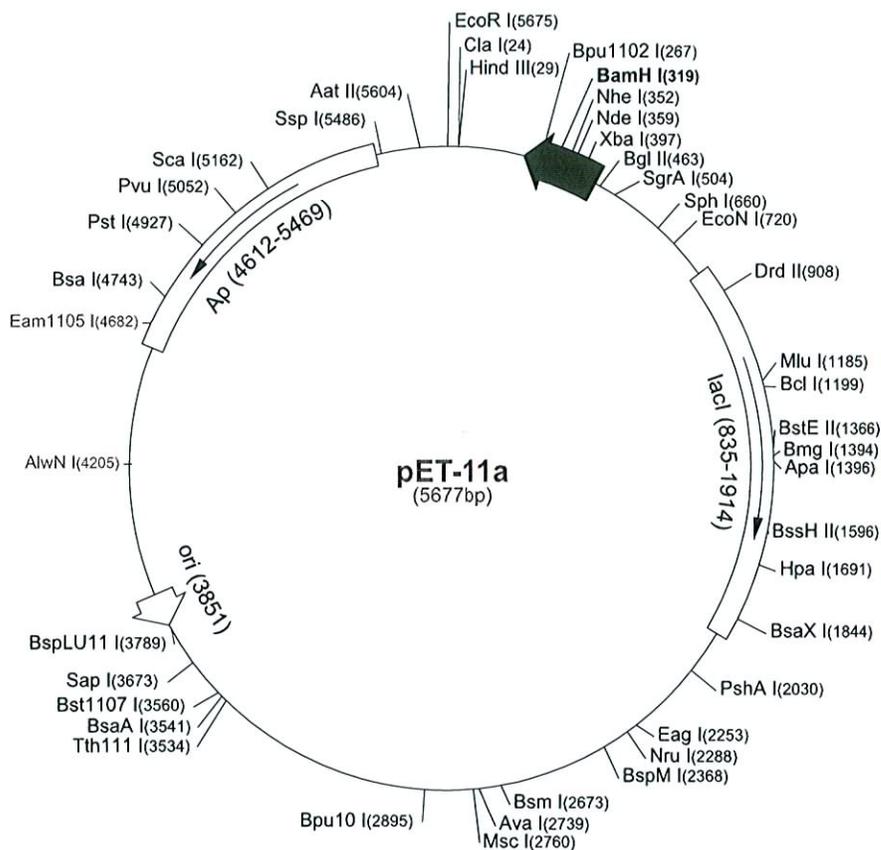
	Cat. No.
pET-11a DNA	69436-3
pET-11b DNA	69437-3
pET-11c DNA	69438-3
pET-11d DNA	69439-3

The pET-11a-d vectors carry an N-terminal T7•Tag<sup>®</sup> sequence and *Bam*H I cloning site. These vectors are the precursors to many pET family vectors; the pET-21a-d(+) series corresponds to pET-11a-d but incorporates several additional features. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below.

### pET-11a sequence landmarks

T7 promoter	432-448
T7 transcription start	431
T7•Tag coding sequence	328-360
T7 terminator	213-259
<i>lac</i> I coding sequence	835-1914
pBR322 origin	3851
<i>bla</i> coding sequence	4612-5469

The maps for pET-11b, pET-11c and pET-11d are the same as pET-11a (shown) with the following exceptions: pET-11b is a 5676bp plasmid; subtract 1bp from each site beyond *Bam*H I at 319. pET-11c is a 5675bp plasmid; subtract 2bp from each site beyond *Bam*H I at 319. pET-11d is a 5674bp plasmid; the *Bam*H I site is in the same reading frame as in pET-11c. An *Nco* I site is substituted for the *Nde* I site with a net 1bp deletion at position 359 of pET-11c. As a result, *Nco* I cuts pET-11d at 355. For the rest of the sites, subtract 3bp from each site beyond position 360 in pET-11a. *Nde* I does not cut pET-11d.



T7 promoter primer #69348-3

→ T7 promoter
→ lac operator
→ Xba I
→ rbs

Bgl II
Nde I
Nhe I
T7•Tag
pET-11a
BamH I
Bpu1102 I

AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGAAATTTGTGACGGGATAACCAATTCCTCTAGAAAATAATTTGTGTTAACTTTAAAGAGGAGA  
 TATACATATGSC TAGCATGACTGGTGGACAGCAAAATGGTCCGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGCTGCTGCCACCCCTGAGCAATAACTAGCATAA  
 MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGlyLysEnd

T7 terminator primer #69337-3

pET-11a Nco I TACCATGGCTAGC MetAlaSer...	pET-11b ...GGTCGGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCCCTGAGCAATAACTAGCATAA ...GlyArgAspProAlaAlaAsnLysAlaArgLysG uAlaGluLeuAlaAlaAlaThrAlaG uGlnEnd	pET-11c,d ...GGTCGGATCCGGCTGCTAACAAAGCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCCCTGAGCAATAACTAGCATAA ...GlyArgIleArgLeuLeuThrLysProGluArgLysLeuSerTrpLeuLeuProProLeuSerAsnAsnEnd	
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T7 terminator

CCCCCTGGGGCCICTAAACGGGCTCTTGAGGGCTTTTGTG

pET-11a-d cloning/expression region