



**Please explain the biological agents and/or biohazardous substances used and how they will be**

An *hsp72* construct, UI-R-CM0-bji-h-10-0-UI.s1 (NCBI Accession Code: BF395145.1), donated by its creators, Bonaldo *et al.* 1996 of the Cancer Biology & Epigenomics Program at Children's Memorial Research Center, 2300 Children's Plaza, Box 220, Chicago, IL 60614-3394, USA, contained a 496 b (base), 65% C/G (cytosine/guanine) content insert which aligns with *hsp72* transcript along a region confined to 43 b of the 5' UTR (untranslated region) and 453 b of the ORF (open reading frame); 99% identity, 100% coverage, <1% gaps, expect= 0. The *hsp72* construct insert was obtained from a Sprague-Dawley, myocardial cDNA (complementary DNA) library, and ligated into pT7T3-Pac plasmid-vector between EcoRI and NotI using polyadapters. pT7T3-Pac is the same vector as pT7T3, (NCBI Accession Code: U13871) except for the use of different polyadapters. (Malone J.A. 1994, direct NCBI submission) pT7T3-Pac is competent cell compliant, and codes for ampicillin resistance via *ampR* gene. pT7T3-Pac contains T7 and T3 promoter sequences 55 bp, 5' & 33 bp, 3' to the *hsp72* insert on the coding strand of the plasmid respectively. The *hsp72* construct was transformed into XL1-Blue competent and then cloned in 4 ml of LB broth. The plasmid was then isolated, digested, and the sequence of interest *in vitro* transcribed as a DIG-labelled riboprobe for *in situ* hybridization on skeletal muscle cross-sections.

All liquids associated with production of the probe were poured into bleach and then properly disposed of. All solid waste was autoclaved before disposal in properly labeled garbage. It should be noted that all of the above was accomplished in a properly certified lab outside of our lab. All that ever returns to our facility is the probe ready for use for *in situ* hybridization.

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

We have three funded research projects underway in our laboratories. One is examining the effect of exercise on the metabolic and cardiovascular consequences of exercise. We are using functional (vascular myography and Langendorff preparation) histochemical and biochemical techniques to determine if exercise is able to delay the progress of vascular disease in diabetics or indeed to rescue specific pathological conditions. Of particular interest is the role played by heat shock proteins in this process. It is in this regard where the specific use in situ hybridization is of value as it allows us to assess which tissues turn on a potential response, shortly after an exercise bout.

A second project is examining the effect of exercise and ginseng on a variety of variables. We will also be making vascular measures in these experiments and intend to determine the potentially protective effect on ginseng on reducing the inflammatory response associated with diabetes. We also intend in this experiment to determine if ginseng has any ergogenic properties with regard to exercise.

Lastly, we will still be using a diabetic model but the intent will be to examine the changes in nerve blood flow in diabetics and determine whether such changes could be associated with the common diabetic neuropathy. We will be using some of the same measures as those noted in the first set of experiments, however, we do not anticipate the use of in situ hybridization at this time.

## 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:

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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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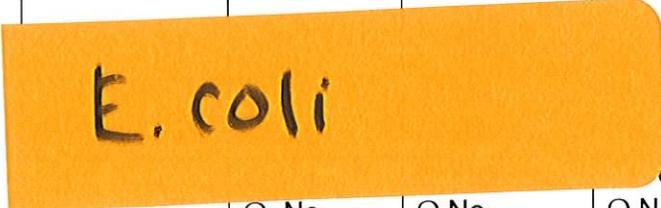


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1.2 Please complete the table below:

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
XLI-Blue	<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	4 ml	Work done in other lab who has already purchased	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
 E. coli	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<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 type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> 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 type="radio"/> Yes <input type="radio"/> No			
Rodent	<input type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Other (specify)	<input type="radio"/> Yes <input type="radio"/> 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

### 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
XL1-Blue	pT7T3, (NCBI Accession Code: U13871; <a href="http://www.ncbi.nlm.nih.gov/nuccore/U13871">http://www.ncbi.nlm.nih.gov/nuccore/U13871</a> )	Uncertain as housed in the lab of colleague	UI-R-CM0-bji-h-10-0-UI.s1 (NCBI Accession Code: BF395145.1)	NA

\* 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 \_\_\_rodent\_\_\_\_\_

6.3 AUS protocol # \_\_\_2008-095; 2010-212\_\_\_\_\_

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 LD<sub>50</sub> (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](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:  
\_\_\_\_\_  
\_\_\_\_\_

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:

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### 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  "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:

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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 \_\_\_\_\_

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.  
 \_\_NA\_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

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:  
 \_\_NA\_\_\_\_\_  
 \_\_\_\_\_

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: Feb 13, 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:



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 consider 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       | • DH5-alpha        | • 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 |
| • BL21 (DE3)  | • EZ10             | • KA802   | • RV308           | • XL0LR        |
| • BM25.8      | • FDA Seattle 1946 | • KAM32   | • S17-1A -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

## Nucleotide

Alphabet of Life

## Section 4

Display Settings: GenBank

## pT7T3D cloning vector, complete sequence

GenBank U13871.1

[FASTA](#) [Graphics](#)[Go to:](#)

LOCUS XXU13871 2907 bp DNA circular SYN 07-DEC-1994  
 DEFINITION pT7T3D cloning vector, complete sequence.  
 ACCESSION U13871  
 VERSION U13871.1 GI:595779  
 KEYWORDS T7 promoter; T3 promoter; beta-lactamase; lacZ alpha peptide.  
 SOURCE unidentified cloning vector  
 ORGANISM [unidentified cloning vector](#)  
 other sequences; artificial sequences; vectors.

REFERENCE 1 (bases 1 to 2907)  
 AUTHORS Malone, J.A.  
 TITLE pT7T3D: A multifunctional directional cloning vector with functions for single-stranded DNA production and in vitro transcription of cloned genes  
 JOURNAL Unpublished  
 REFERENCE 2 (bases 1 to 2907)  
 AUTHORS Malone, J.A.  
 TITLE Direct Submission  
 JOURNAL Submitted (19-AUG-1994) James A. Malone, International Technical Services, Molecular Biology Reagents Division, Pharmacia Biotech Inc., 2202 N. Bartlett Ave., Milwaukee, WI 53202-1009, USA

FEATURES  
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[-10 signal](#)  
 complement(711)  
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