

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.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

PRINCIPAL INVESTIGATOR	Earl Noble
DEPARTMENT	Kinesiology
ADDRESS	2170 Thames Hall
PHONE NUMBER	88365 Office; 88373 lab
EMERGENCY PHONE NUMBER(S)	519-472-8399; 519-870-8399
EMAIL	enoble@uwo.ca

*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).

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

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:

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
XLI-Blue	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	4 ml	Work done in other lab who has already purchased	☒ 1 ☐ 2 ☐ 2+ ☐ 3
E. coli	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐ YES ☒ 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		☐ 1 ☐ 2 ☐ 2+ ☐ 3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐ 1 ☐ 2 ☐ 2+ ☐ 3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐ 1 ☐ 2 ☐ 2+ ☐ 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; http://www.ncbi.nlm.nih.gov/nuccore/U13871)	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 _____ ☒ YES ☐ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☐ YES, specify _____ ☒ YES ☐ NO
- ◆ SV 40 Large T antigen ☐ YES ☒ YES ☐ NO
- ◆ E1A oncogene ☐ YES ☒ YES ☐ NO
- ◆ Known oncogenes ☐ YES, please specify _____ ☒ YES ☐ NO
- ◆ Other human or animal pathogen and or their toxins ☐ YES, please specify _____ ☒ YES ☐ 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 ☒ 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 LD₅₀ (specify species) of the toxin _____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? ☐ YES, Please provide details: _____ ☐ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? ☐ YES ☒ NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

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 ☐ "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 _____

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:



Canadian Food Inspection Agency
Agence canadienne d'inspection des aliments



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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

• 5K	• 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,

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

Canada

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
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 promoter 172..191
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 promoter complement(275..294)
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1441  agtatcatatg agtaaacctt gttccgcagt taaccaatgtt taatcagtga ggcacctatc
1501  tcagcagatct gtttatcttg tttatccata gttgtctgac ccccgctctt gtagataact
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1921  agaagttaagt tggccgcagt gttatccact atggttatgt cagcacttga taactctctt
1981  accgtcactgt cactccgttaag atgctttctt gtagctgtgt agtactcaac caagtcatct
2041  ttagaataagt gttatcggcg accgagttct tcttgcccg ggtcaatacg ggaataatcc
2101  ggcgcacata gtagaacttt aaaaagtctt atcattggaa aacgttcttc gggcgaaaaa
2161  ccttcaaggc ttttacccgt gtcgagatcc agttcagatg aacccactct tgcacccaaa
2221  tcatcttcag catctttttac tttcacccag gttctcgggt cagcaaaaaa aggaaggcaa
2281  aatgcgcgcaa aaaaagggaat aaggcgagca cggaaatgtt gaataactcat actcttcttt
2341  tttcaatatc attgaaagcat cctcaggggt taattgtctc ttagcgggata catatttgaa
2401  tgtatttcaga aaaataaaaa aataggggtt ccgcgcacat ttcccgaaa agtcaccaat
2461  gaaatcgttaa accgttaactt tttgtcaaat cccgcgtcaa atatttgtta aatcagctta
2521  tttttcaaac agtcaagcaga aaatgacaaa aatccctaca aatcaaaaga atagacccag
2581  tttagctgtga cgtctgtttc agtttggaa cagagtcac ctttaaaaga cgtggacttc
2641  aacgttaaaa cgtctatcag ggcgatggcc cactacgtga accacacccc aaatcaagtt
2701  tctggaggctt gagggtccctt aaagcactaa accggaaccc taaggggag ccccgactta
2761  gactctgagc gggaaagcgc gcaacgcgg cagaaaaagg agggaaagaa cgcgaaggag
2821  cgggcgctag ggcgcgggca agtctagcgt tcaacgtcgt cgttaacccc acccccgcg
2881  cgttcaatgc cccgctactc ggcgcgtt

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