THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

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

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Shengwu Ma
DEPARTMENT	Biology
ADDRESS	346 South Street
PHONE NUMBER	519-685-8500 ext. 75855
EMERGENCY PHONE NUMBER(S)	519-639-7805
EMAIL	sma@uwo.ca

Location of experimental work to be carried out: Building(s) 346 Victoria Health Services Building _____ Room(s) 265

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): Use of plants and algae to express and deliver inexpensive human proteins for the treatment of type 1 diabetes

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

Name	UWO E-mail Address	Date of Biosafety Training
Jeehye Choi	Jchoi3@uwo.ca	08/2010
Hong Diao	hdiao@uwo.ca	08/2010

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.

Bacterial strains used:

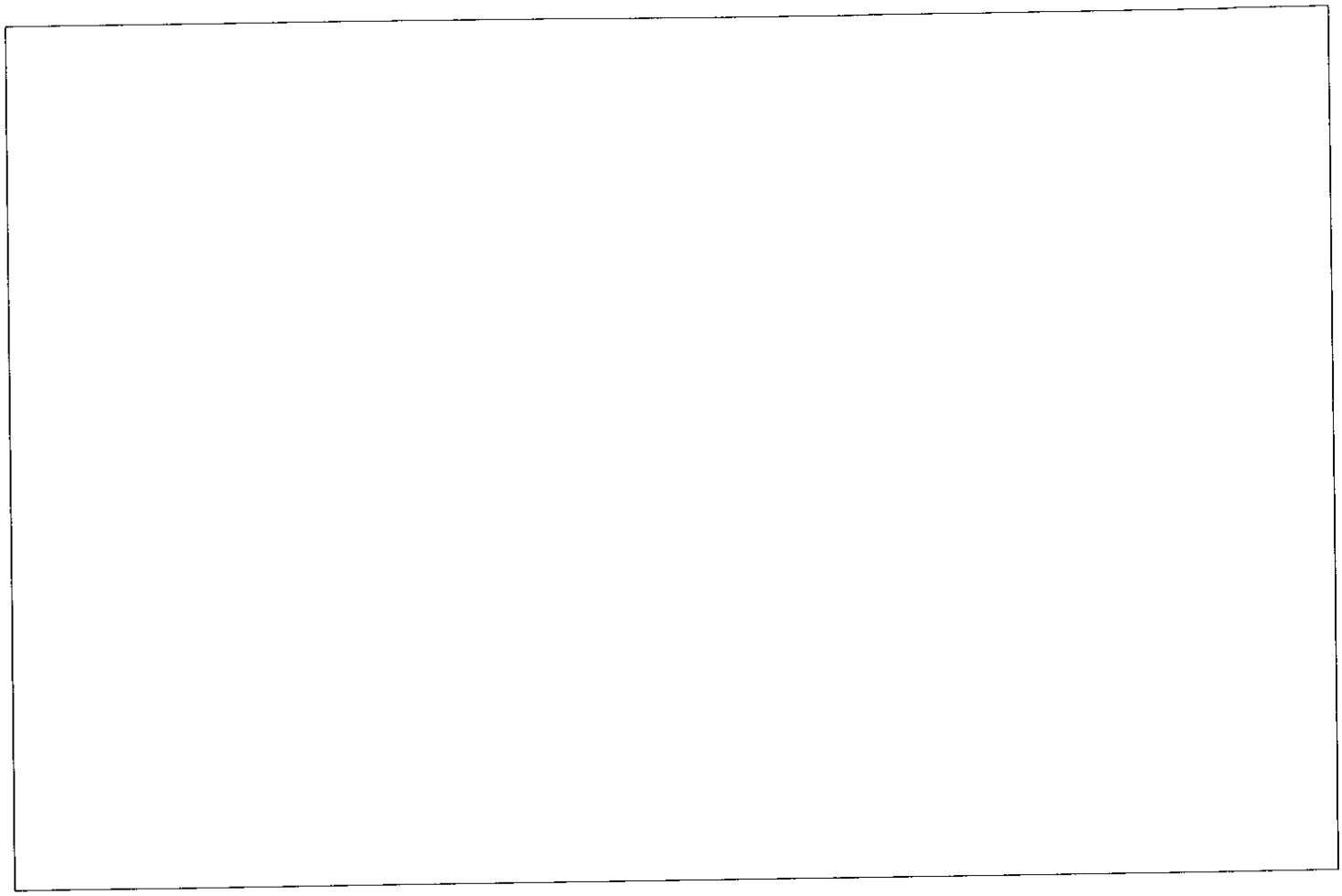
1. E. coli DH5α — the most commonly used bacterial strain in my laboratory. It is frequently used for plasmid transformation, plasmid preparation and bacterial culture. This bacterial strain is commercially available, non-pathogenic. The bacterial culture is killed by autoclave before disposal.
2. Agrobacterial strains — Agrobacterium tumefaciens LBA4404 is also the most frequently used bacterial strain in my laboratory. It is used in most cases for plant genetic transformation. This bacterial strain is commercially available, non-pathogenic. The bacterial culture is killed by autoclave before disposal.
3. Green Algae — Chlamydomonas reinhardtii is an algal species that is often used in our laboratory. It is used for transgenic transformation. Chlamydomonas reinhardtii is the most widely used laboratory species. It is non-pathogenic. The culture is killed by autoclave before disposal.

Transgenic plants used:

1. Transgenic tobacco — We produce and grow transgenic tobacco plants. Transgenic tobacco plants are grown either in laboratory or in the department's greenhouse. In most cases, these transgenic tobacco plants are grown not beyond the flowering stage. Transgenic plant materials are autoclaved before disposal.
2. Transgenic potato plants. We also produce and grow transgenic potato plants. Transgenic potato plants are grown either in laboratory or in the department's greenhouse. Transgenic plant materials are autoclaved before disposal.

Waste disposal? (hospital)

#3. C. reinhardtii Info on transgenic transformation?



This research aims to develop a novel expression system enabling the inexpensive production of large quantities of human proteins for the treatment of human type 1 diabetes via oral tolerance induction. The innovative system is based on the utilization of the green algal chloroplast as a living factory for recombinant protein production. Accumulating evidence suggests that production of human proteins in algae by means of chloroplast transformation offers distinct advantages over conventional microbial or higher plant expression systems. These include ease and speed of transformation, the ability to achieve very high expression of foreign proteins, and low production cost. Moreover, as algae have been used for years as a source of food in some countries, they can be used as an oral delivery vehicle for the produced therapeutic proteins. The specific objectives of this project are to: 1) produce high levels of the diabetes-associated autoantigens glutamic acid decarboxylase (GAD) and insulin, and 2) produce high levels of immunoregulatory molecules such as cytokine interleukin-4 (IL-4)

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O 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? O YES X 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
E. coli DHα	O Yes X No	O Yes X No	O Yes X No	5 to 25 ml	New England BioLabs	✓ 1 O 2 O 2+ O 3
A.tumefaciens LBA4401	O Yes X No	O Yes X No	O Yes X No	5 to 25 ml	Invitrogen	✓ 1 O 2 O 2+ O 3
C. reinhardtii strain 137C	O Yes X No	O Yes X No	O Yes X No	25 to 100 ml	Chlamydomonas Genetics Center	✓ 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 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? O YES X 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	O Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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	O Yes O No			
Rodent	O Yes O No			
Non-human primate	O Yes O No			
Other (specify)	O Yes O No			

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES X 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		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 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? X YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? X YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
E. coli DHα	*pUC 19*	*New England BioLabs*	*Human glutamic decarboxylase gene (GAD65)*	*No phenotypic changes*
Agrobacterium tumefaciens LBA4404	*pBI 101*	*Clontech*	*GAD65*	*No phenotypic changes*

* 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?
O YES, complete table below X 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 O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen O YES X NO
- E1A oncogene O YES X NO
- Known oncogenes O YES, please specify _____ X NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

4.5 Will virus be replication defective? O YES X NO

4.6 Will virus be infectious to humans or animals? O YES X NO

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X 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? O YES X 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? X YES O NO

See Question 10.6

6.2 Name of animal species to be used_mice_____

6.3 AUS protocol # __AUP# 2008-059_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ X NO

6.5 Will the agent(s) be shed by the animal: O YES X 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)? O YES X No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O 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? O YES X 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_{50} (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? O YES, Please provide details:_____ X 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? O YES X 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? O Initiate and maintain colony, give location: _____
O "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? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? X YESO NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. 'Nicotiana Tabacum (tobaco); Solanum tuberosum (potato)
_____

10.3 What is the origin of the plant? __ American _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _leaves or tubers_____

10.5 What is your intention? O Grow and maintain a crop X "One-time" use

10.6 Do you do any modifications to the plant? X YESO NO
If yes, please describe: Tobacco or potato plants are transformed with plasmids containing human GAD65 gene to develop transgenic plants. Tobacco levels or potato tubers expressing human proteins will be fed to mice to determine if plant-made pharmaceuticals can protect animals from developing type 1 diabetes.
_____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

No incidence of loss of transgenic plant materials from the lab has been reported. The lab door is always kept locked. _____
_____
_____

10.8 Is the CFIA permit attached? O YES Ø 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? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES XNO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES X NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ X 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 Shengde Ma

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: general lab inspection completed Sept. 16/10 Yaire Ryder
○ 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.
Non-pathogenic agents used, therefore N/A. Level 1 work.

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:

Students are required to wear gloves and lab coats to protect them from direct contact with genetically engineered microorganisms such as E. coli or Agrobacterium cells while performing experiments. In case there is a spill of cell culture, clean spills of culture immediately.

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 Shengde Ma Date: June 28, 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: Gail Ryder
Date: June 29, 2011

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Material Safety Data Sheets

MATERIAL SAFETY DATA SHEET (MSDS)

NEW ENGLAND BioLabs Inc.
New England Biolabs
240 County Road
Ipswich, MA 01938

Telephone: (978) 927-5054
Toll free: (800) 632-5227
Fax: (978) 921-1350
Email: info@neb.com
Revision Date: 9/10

NEB #C2987

SECTION 1–CHEMICAL INFORMATION

Product Name: NEB 5-alpha Competent *E. coli* (High Efficiency) Cas.# None

SECTION 2–COMPOSITION/INFORMATION ON INGREDIENT

1.	Glycerol	1–10%	Cas.# 56-81-5
2.	Dimethyl Sulfoxide	1–10%	Cas.# 67-68-5

The ingredients listed in this section include only those items that have more than 1% of a component classified as hazardous and 0.1% of a component classified as carcinogenic. If you have any questions, please contact info@neb.com.

SECTION 3–HAZARDOUS IDENTIFICATION

Emergency Overview: Warning: May cause irritation to skin, eyes, and respiratory tract, may affect kidneys, blood and liver.
HMIS and NFPA Ratings: 0 – Minimal or None, 1 – Slight, 2 – Moderate, 3 – Serious, and 4 – Severe

Health: 1
Flammability: 1
Reactivity: 0

SECTION 4–FIRST AID MEASURES

Eyes: Flush eyes with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating eyelids. Call a physician.
Skin: Wash skin with soap and copious amount of water.
Ingestion: If the person is conscious, wash out mouth with water. Call a physician.
Inhalation: Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SECTION 5–FIRE FIGHTING MEASURES

Extinguishing Media: Water spray. Carbon dioxide, dry chemical powder or appropriate foam.
Special Fire Fighting Procedures: Wear self contained breathing apparatus and protective clothing to prevent contact with skin and eyes.
Fire and Explosion Hazards: Combustible liquid. Emits toxic fumes under fire conditions.

SECTION 6–ACCIDENTAL RELEASE MEASURES

Personal Precautions: Avoid breathing or contact with vapors, mist or gas.
Procedure of Personal Precaution: Wear self-contained breathing apparatus, rubber boots and heavy rubber gloves and chemical safety goggles. Use non-sparking tools and equipment. Ventilate and evacuate area of leak or spill.
Environmental Precautions: Do not let product enter drains.
Methods For Cleaning Up: Cover with dry lime, sand, or soda ash. Sweep up and shovel. Place in covered container for disposal.

SECTION 7–HANDLING AND STORAGE

Handling: Provide appropriate exhaust ventilation.
User Exposure: Avoid inhalation. Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.
Storage: Keep tightly closed in a dry and well ventilated place. Store at -20°C .

Engineering Controls: Safety shower and eye wash. Mechanical exhaust.

Personal Protective Equipment

Eye Protection: Safety goggles.

Hand Protection: Compatible resistant gloves.

Respiratory Protection: Government approved respirator.

Hygiene Measure: General practice, wash (hands and skin) thoroughly after handling. Remove and wash contaminated clothing.

Physical State: Form: Liquid Color: Clear or colorless Odor: No Data Available

Property	Value	Temperature or Pressure	
Boiling Point Range:		>189°C	
Melting Point Range:		>18.4°C	
Flash Point:		>87°C	Method: Closed cup
Auto Ignition Temp:		>215°C	
Vapor Pressure:	.42 mmHg	20°C	
Vapor Density:	2.7 g/l		
Specific Gravity:	1.1		
Solubility in Water:	Soluble		

Stability: Stable under recommended storage conditions.

Conditions to Avoid: Moisture

Materials to Avoid: Acid chlorides, Phosphorus halides, strong oxidizing agents, strong acids, strong reducing agents.

Hazardous Decomposition Products: Carbon monoxide, Carbon dioxide, Sulfur dioxides.

Hazardous Polymerization: Will not occur.

Hazardous Exothermic Reactions: Hazardous Exothermic Reactions: Methyl sulfoxide (DMSO) undergoes a violent exothermicreaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phorphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver flioride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride and perchloric and periodic acids. When heated above its boiling point methyl sulfoxide degrades giving off formaldehyde, methyl mercaptan and sulfur dioxide.

Acute and Chronic Affects Based On Routes Of Exposure

Effects on Fertility: Pre-implantation mortality (e.g., reduction in number of implants per female; total number of implants per corpora lutea).
Effects on Embryo or Fetus: Fetotoxicity (except death, e.g., stunted fetus).

Specific Developmental Abnormalities: Musculoskeletal System

Eye Contact: May cause irritation.

Skin Contact: May cause irritation.

Ingestion: May cause nausea, coughing, headache or diarrhea.

Inhalation: Unlikely at room temperature, inhalation of mist may cause irritation of respiratory tract.

Chronic Exposure

Target Organ(s): May cause kidney and liver damage.

Aggravation of Pre-existing Conditions: Persons with pre-existing skin disorder or eye problems or impaired liver or kidneys may be more susceptible to the effects of the material.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen.

IARC: No component of this product present at levels greater than or equal to 0.1 % is identified as probable, possible or confirmed human carcinogen.

ACGIH: No component of this product present at levels greater than or equal to 0.1 % is identified as a known or suspected human carcinogen or confirmed animal with unknown relevance humans.

Route of Exposure
Skin Absorption: May be harmful if absorbed.
Contact: May cause skin irritation.
Eye Contact: May cause eye irritation.
Inhalation: May be harmful if inhaled. Material may be irritating to mucous membranes and upper respiratory tract.
Ingestion: May be harmful if swallowed.
Conditions Aggravated By Exposure: Avoid contact with DMSO solutions containing toxic materials or material with unknown toxicological properties. Dimethyl sulfoxide is readily absorbed through skin and may carry such materials into the body. Avoid prolonged or repeated exposure.

Target Organ (s) or System (s): Eyes and Skin

Toxicity Data

Inhalation
Rat
40,250 ppm
LC50

Oral
Rat
3,300 mg/kg
LD50

Oral
Rat
14,500 mg/kg
LD50

Remarks: Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Hemorrhage. Sense Organs and Special Senses (Nose, Eye, Ear and Taste): Eye: Conjunctive irritation.

Skin
Rat
40,000 mg/kg
LD50

Intraperitoneal
Rat
8,200 mg/kg
LD50

Subcutaneous
Rat
12 gg/kg
LD50

Remarks: Behavorial: Change in motor activity (specific assay), Lungs, Thorax, or Respiration: Dyspnea.

Intravenous
Rat
5,360 mg /kg
LD50

Remarks: Behavorial: Tremor, Muscle weakness. Lungs, Thorax or Respiration: Dyspnea.

Chronic Exposure - Carcinogen
Species: Rat
Route of Application: Oral
Dose: 59 gm/kg
Exposure Time: 81W
Frequency: I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Species: Rat
Route of Application: Subcutaneous
Dose: 220 gm/kg
Exposure Time: 82W
Frequency I

Result: Tumorigenic: Equivocal tumorigenic agent by RTECS criteria, Skin and Appendages: Other: Tumors.

Chronic Exposure - Mutagen
Species: Rat
Route: Intraperitoneal
Dose: 25 gm/kg
Exposure Time: 5D
Mutation Test: Cytogenetic analysis.

Chronic Exposure - Reproductive Hazard
Species: Rat
Dose: 56 gm/kg
Route of Application: Intraperitoneal
Exposure Time: (6–12D PREG)
Result: Effects on Fertility: Abortion

Species: Rat
Dose: 6,600 mg/kg
Route of Application: Intraperitoneal
Exposure Time: (7–15D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants).

Species : Rat
Dose: 30,750 mg/kg
Route of Application: Subcutaneous
Exposure Time: (8–10D PREG)
Result: Effects on Fertility: Post-implantation mortality (e.g., dead and/or resorbed implants per total number of implants). Effects on Fertility: Litter size (e.g.; # fetuses per litter; measured before birth).

Elimination Information (persistence and degradability): No data available.

Ecotoxicity Effects

Toxicity to fish	LC50-Pimephales promelas (fathead minnow) - 34,000 mg/l - 96 h
	LC50-Oncorhynycus mykiss (rainbow trout) - 35,000 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	EC50-Daphnia pulex (water flea) - 27,500 mg/l
Toxicity to algae	EC50 - Lepomis macrochirus (Blue Gill) - > 400,000 mg/l - 96 h

Further Information On Ecology: No data available.

Dispose of container, unused contents and contaminated packaging in accordance with federal, state and local requirement. Contract with a licensed Chemical Waste Disposal Service.

This product is not dangerous and no special precautions are needed according to DOT, ADR/RID (cross border), IMDG and IATA/ICAO.

OSHA Hazards: None known.

US Classification and Label Test

US Statements: Combustible. Readily absorbed through skin. Target Organ (s): Eyes, skin, liver and kidneys. Caution. Avoid contact and inhalation.

United States Regulatory Information:

Sara Listed: No

TSCA Inventory Item: Yes

Canada Regulatory Information

WHMIS Classification: This product has been classified in accordance with the hazard criteria of the CPR and the MSDS contains all the information required by the CPR.

DSL: Yes

NDSL: No

EU Additional Classification

S: 23 24/25

Safety Statements: Do not breath vapor. Avoid contact with skin and eyes.

DISCLAIMER

The information provided on the MSDS is furnished in good faith and based on our present knowledge. However, this MSDS shall not constitute a guarantee of any kind. Personnel handling this material must make independent determinations of the suitability and completeness of information from all sources to assure proper use and disposal of this material and the safety and health of employees and customers. NEB assumes no additional liability or responsibility resulting from the use of, or reliance on this information. This product is for R&D use only. Not for drug, household or other uses.

Questions about the information found on this MSDS should be directed to info@neb.com.

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 consider 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
- XLOLR
- 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

Canada

invitrogen™

Certificate of Analysis

ElectroMAX™ *Agrobacterium tumefaciens*
LBA4404 Competent Cells

Part No./Catalog No. 18313015
Lot Number: 1395970

Transformation Efficiency

20 µl of competent cells are transformed with 5 ng of pBI121 plasmid DNA (non-saturating conditions). Test transformations are performed on a minimum of 3 vials per lot. Transformed cultures are plated on YM medium plates containing 100 µg/ml streptomycin and 50 µg/ml kanamycin and incubated at 30°C for 48–72 hours.

Transformation efficiency must be greater than 5.0×10^6 cfu/µg pBI121.

Antibiotic Sensitivity

(All plates are incubated at 30°C unless otherwise noted.)

Cells must exhibit growth on YM medium plates.

Untransformed cells must show no growth on YM plates containing 100 µg/ml streptomycin and 50 µg/ml kanamycin, indicating the absence of any kanamycin resistance markers. (Streptomycin is added for selection of the pAL 4404 plasmid.)

Cells should exhibit growth on LB medium plates incubated at 30°C

Cells should show inhibited growth on LB medium plates incubated at 42°C

Untransformed cells must show no growth on LB plates containing 100 µg/ml ampicillin, indicating the absence of any ampicillin resistance markers.

Untransformed cells must show no growth on LB plates containing 50 µg/ml kanamycin, indicating the absence of any kanamycin resistance markers.

Untransformed cells must show no growth on LB plates containing 15 µg/ml tetracycline, indicating the absence of any tetracycline resistance markers.

Untransformed cells must exhibit growth on LB plates containing 100 µg/ml streptomycin, indicating the presence of streptomycin resistance markers.

Gal Phenotype

Cells must exhibit growth of white or light pink colonies on MacConkey galactose plates, indicating a Gal $^-$ phenotype.

Absence of Bacteriophage

To verify the absence of phage contamination, 0.5–1.0 ml of LBA4404 competent cells are added to LB top agar along with *E. coli* lawn cells and poured over LB plates. After incubation at 30°C for 48–72 hours, no plaques should be detected.

Results

Product meets all specifications.

All products are for research use only. Caution: Not intended for human or animal diagnostic or therapeutic uses. If you have any further questions about this Certificate of Analysis, please contact Technical Services at 1-800-955-6288 (US and Canada) or 1-760-603-7200, x2 (all other countries).

Kel Locklar

Kel Locklar
Senior Manager, Quality Systems

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

Good Morning,

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

Thank you,
Steven Burns

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

CC-124 wild type mt- 137c

C. reinhardtii
strain 137c

llele nit1-137
llele nit2-137

ource: R.P. Levine via N.W. Gillham, 1968

:omment: This is the basic "137c" wild type strain originally from G.M. Smith, isolated in 1945 near Amherst MA, nd is presumably equivalent to strain 11/32d of the Culture Centre of Algae and Protozoa. This particular strain was rought to Duke by N.W. Gillham in 1968 from Levine's laboratory at Harvard. CC-124 and CC-125 carry the nit1 nd nit2 mutations, and cannot grow on nitrate as their sole N source. CC-124 carries the agg1 allele for phototactic ggregation; see *The Chlamydomonas Sourcebook* [Harris (1989)], p. 215. Contrast CC-125, which has the agg1+ llele at this locus.

:eturn to : [core collection list | [on-line order form | Chlamydomonas Center]

lablife

Community

Plasmid(s)

Vector Database > pBI101

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pBI101
Source/Vendor	Clontech
Plasmid Size	5350
Notes	Hosts: E.coli, plant. Related vectors: pBIN19, pBI101.2, pBI101.3. (Information source: VectorDB.)
Link	http://seq.yeastgenome.org/vectordb/vector_descrip/PBI101.html
Plasmid Sequence	View Sequence

pUC19

GenBank Accession #: L09137

Feature	Coordinates	Source
lacZα	469-146	--
origin	1455-867	pMB1 (mutant)
bla (ApR)	2486-1626	*Tn3*

ori = origin of replication
Ap = ampicillin

There are no restriction sites for the following enzymes: AarI(x), AfeI, AflII, AgeI, AleI, ApaI, AscI, AsiSI, AvrII, BaeI, BbsI, BbvCI, BclI, BglII, BlpI, BmgBI, BmtI, Bpu10I, BsaAI, BsaBI, BseRI, BsgI, BsiWI, BsmFI, BsmI, BspDI, BspEI, BsrGI, BssHII, BstBI, BstEII, BstXI, BstZ17I, Bsu36I, BtgI, BtgZI, ClaI, CspCI, DraIII, EagI, EcoNI, EcoRV, FseI, FspAI(x), HpaI, I-CeuI, I-SceI, MfeI, MluI, MscI, NacI, NcoI, NgoMIV, NheI, NotI, NruI, NsiI, PI-PspI, PI-SceI, PacI, PaeR7I, PflFI, PflMI, PmeI, PmlI, PpuMI, PshAI, PsiI, PspOMI, PspXI, RsrII, SacII, SanDI(x), SexAI, SfiI, SgrAI, SnaBI, SpeI, SrfI(x), StuI, StyI, SwaI, TliI, Tth111I, XcmI, XhoI

(x) = enzyme not available from NEB

pUC19 is a small, high-copy number *E. coli* plasmid cloning vector containing portions of pBR322 and M13mp19 (1). It contains the pMB1 origin of replication from pBR322, but it lacks the *rop* gene and carries a point mutation in the RNAII transcript (G 2975 in pBR322 to A 1308 in pUC19; 2). These changes together result in a temperature-dependent copy number of about 75 per cell at 37°C and >200 per cell at 42°C (2, 3). The multiple cloning site (MCS) is in frame with the *lacZα* gene, allowing screening for insertions using α-complementation.

pUC18 is identical to pUC19 except that the MCS region (nt 397-454) is inverted.

pNEB193 is also identical to pUC19 except for the addition of several restriction endonuclease sites to the MCS. Its total length is 2713 bp.

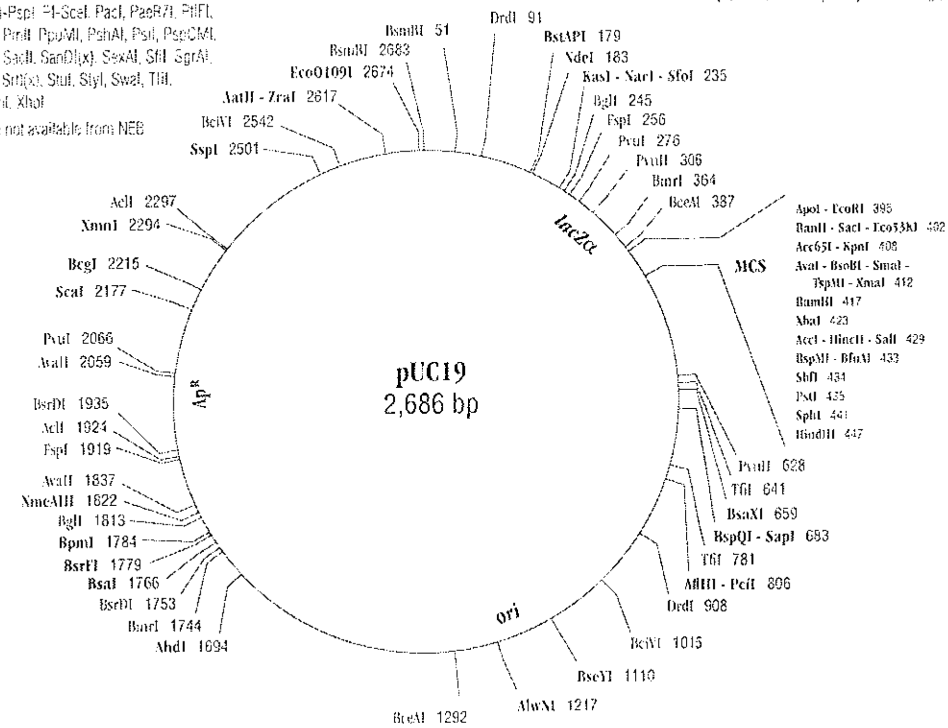
Enzymes with unique restriction sites are shown in **bold** type, and enzymes with two restriction sites are shown in regular type. Location of sites of all NEB restriction enzymes can be found on the NEB web site (choose Technical Reference > DNA Sequences and Maps). Restriction site coordinates refer to the position of the 5´-most base on the top strand in each recognition sequence.

Open reading frame (ORF) coordinates are in the form: "translational start – translational stop"; numbers refer to positions on the top (clockwise) strand, regardless of the direction of transcription and include the start and stop codons.

Origin of replication coordinates include the region from the -35 promoter sequence of the RNAII transcript to the RNA/DNA switch point. *bla* (ApR) gene coordinates include the signal sequence.

References

(1) Yanisch-Perron, C., Vieira, J. and Messing, J. (1985) *Gene*, 33, 103–119.
(2) Lin-Chao, S., Chen, W.-T. and Wong, T.-T. (1992) *Mol. Microbiol.*, 6, 3385–3393
(3) Miki, T. et al. (1987) *Protein Eng.*, 1, 327–332.



pUC19 MCS

```
            SacI         SmaI          XbaI         SbfI
    EcoRI          KpnI        BamHI           SalI  PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat

      400       410       420       430       440       450       460

...S  N  S  S  P  V  R  P  D  E  L  T  S  R  C  A  H  L  S  P  T  I  M  T  M
```

← *lacZα* translational start

pNEB193 MCS

```
          SacI       SmaI   BssHII       PacI                          SbfI
    EcoRI      KpnI       AscI    BamHI          XbaI  SalI   PmeI    PstI  SphI  HindIII
agtgAATTCGAGCTCGGTACCCGGGGCGCGCCGGATCCTTAATTAAGTCTAGAGTCGACTGTTTAAACCTGCAGGCATGCAAGCTTGGcgtaatcatggtcat

      400       410       420       430       440       450       460       470       480       490

...S  N  S  S  P  V  R  P  R  A  P  D  K  I  L  D  L  T  S  Q  K  F  R  C  A  H  L  S  P  T  I  M  T  M
```

← *lacZα* translational start