

Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

Approved Personnel

(Please stroke out any personnel to be removed)

~~Jen-Mepham~~

Jessica Kao

~~Katie Moisse~~

Cheryl Leystra-Lantz

Wendy Strong

May Gohar

Wencheng Yang

Kathy Volkening

Cristian Droppelmann

Additional Personnel

(Please list additional personnel here)

Zheng Ping He

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Sep 7, 2007

Signature of Permit Holder



Aug 26-09

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli (DH5 alpha, XL1 Blue, XL10 Gold), Att109 yeast, Y187 yeast	E. coli BL21 (DE3)
Approved Cells	Rodent (primary)m Human (established), Rodent (established), IMR32/HEK-293T, Neuro 2A, pc12, EOC20, NSC34, BV2, LADMAC, human cortical neurons: HCN-1A	
Approved Use of Human Source Material	brain, spinal cord	
Approved GMO	SV 40 Large T antigen, E1A	
Approved use of Animals		
Approved Toxin(s)		

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Classification: 2

Date of last Biohazardous Agents Registry Form: Sep 7, 2007

Signature of Permit Holder _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Subject: BL21
From: lantz@robarts.ca
Date: Tue, 25 Aug 2009 14:50:58 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Quite simply, the BL21 (DE3) strain of *E. coli* is a protease-deficient strain of bacteria used for the high level expression of mammalian recombinant proteins.

I hope that this is sufficient for our protocol BIO_RRI-0035.

Cheers,
Cheryl

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 500149
Product name BL21 (DE3) One Shot

Company/Undertaking Identification

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

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

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

24 hour Emergency Response (Transport): 866-536-0631
 301-431-8585
 Outside of the U.S. ++1-301-431-8585

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	10-30

3. HAZARDS IDENTIFICATION

Emergency Overview

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

Form
 Liquid

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m ³ total dust 5 mg/m ³ respirable fraction	-	10 mg/m ³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

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

10. STABILITY AND REACTIVITY

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral, rat/mouse)	LD50 (dermal, rat/rabbit)	LC50 (inhalation, rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m ³ (Rat)

Principle Routes of Exposure/

Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available
Ingestion No information available

Specific effects

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

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

16. OTHER INFORMATION

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

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

Approved Personnel

(Please stroke out any personnel to be removed)

Jen Mephram
Jessica Kao
Katie Moisse
Wendy Strong
May Gohar
Wencheng Yang
Kathy Volkening

Additional Personnel

(Please list additional personnel here)

ZhongPing He
Cristian Droppelmann
Cheryl Leystra-Lantz

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli (DH5 alpha, XL1 Blue, XL10 Gold), Att109 yeast, Y187 yeast	
Approved Cells	Rodent (primary)m Human (established), Rodent (established), IMR32/HEK-293T, Neuro 2A, pc12, EOC20, NSC34, BV2, LADMAC, human cortical neurons: HCN-1A	
Approved Use of Human Source Material	brain, spinal cord	
Approved GMO	SV 40 Large T antigen, E1A	

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Sep 7, 2007

Signature of Permit Holder:

 Mike Strong

BioSafety Officer(s): Stanley

June 26/09

Chair, Biohazards Subcommittee:

 Stanley

Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

Approved use of
Animals

Approved Toxin(s)

Plasmids : see list

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Classification: 2

Date of last Biohazardous Agents Registry Form: Sep 7, 2007

Signature of Permit Holder: See page 1

BioSafety Officer(s): V. Tanley June 26/09

Chair, Biohazards Subcommittee: S. K. Kildner

The plasmids in Dr. Michael Strong's lab are used to transform non-pathogenic *E. coli* (for growth of multiple copies of the plasmid). Final plasmids recovered are used to either produce RNA or protein *in vitro* for *in vitro* binding assays, backbones for RT-PCR studies or to transfect cell lines to study expression patterns and interactors.

Plasmid	Plasmid
pCMX	pTRLamp
pGEX-2T	pRSVi
pGEX-4T2	pRK172
pGEX5X-3	pBluescript SK(-)
pAS2-1	pT7T3D
pCR-XL-TOPO	pOTB7
pEII-TOPO	pGEM-Teasy
pcDNA3.1(+)	pGEM4Z
pcDNA-BrA	pGEM7Zf
pcDNA3.1-myc hisA	pGBKT7-53
pcDNA3.1-myc hisB	pGBKT7-LAM
pcDNA3.1-myc hisC	pGBKT7-DNA-BD
pcDNA/CMV-HA ubiquitin	pCMV-SPORT6
pRFP-N1	pGAD-424
pRFP-N2	pGAD-T7
pRFP-N3	pAG306Gal ⁻ ccdB-EGFP
pEYFP-N1	
pEYFP-C1	
pIRES-EGFP	
pBridge	
pEGFP-C1 (new)	
pEGFP-N1	
pECFP-C1	
pSuper	
pShrek-GFP.BAP	
pShrek-hTRA2.BAP	
pShrek-BABP.BAP	

pAG306Gal⁻ccdB-EGFP → see email attached

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

Subject: Re: New MTA for M. Strong lab plasmid request (Order 26086)

Date: Fri, 29 May 2009 12:09:53 -0400

From: lantz@robarts.ca

To: Jennifer Stanley <jstanle2@uwo.ca>

References: <0KKD00LJ82FQPZ10@zeppo.mail.uwo.pri>

<4A1EDF25.5040902@uwo.ca>

<200905291310.n4TDAqWa020495@doom.robarts.ca>

<4A20040F.40708@uwo.ca>

It was the plasmid **before** this order that I included on the list, sorry. Please add pAG306Gal-ccdB-EGFP.

Thanks,
Cheryl

Modification Form for Permit BIO-RRJ-0035

Permit Holder: Mike Strong

Approved Personnel
(Please stroke out any personnel to be removed)

~~Saima Humayun~~
Kate Moisse
Wendy Strong
May Gohar
Wencheng Yang
Kathy Volkoning

Additional Personnel
(Please list additional personnel here)

Jessica Kao
Jan Mephum

Approved Microorganisms

Please stroke out any approved Biohazards to be removed below

E. coli (DH5 alpha, XL1 Blue, XL10 Gold)

Write additional Biohazards for approval below. *

Att1091 yeast
Y187 yeast

Approved Cells

Rodent (primary)m Human (established),
Rodent (established), IMR32/HEK-293T,
Neuro 2A, pc12, EOC20, NSC34, BV2,
LADMAC

human cortical neurons:
HCN-1A

Approved Use of Human Source Material

brain, spinal cord

Approved GMO

SV 40 Large T antigen, E1A

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

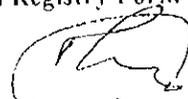
** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Date of last Biohazardous Agents Registry Form Sep 7, 2007

Signature of Permit Holder:

BioSafety Officer(s):

Chair, Biohazards Subcommittee:


Sept 30/08


Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

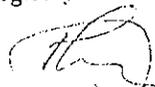
Approved use of
Animals

Approved Toxin(s)

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

Date of last Biohazardous Agents Registry Form Sep 7, 2007

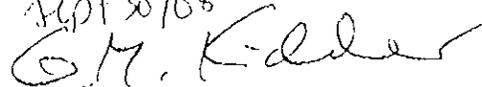
Signature of Permit Holder:



BioSafety Officer(s):

W. H. Hines Sept 30/08

Chair, Biohazards Subcommittee:



Description of work involving strains AH109 and Y187 yeast:

The AH109 and Y187 strains of yeast will be obtained as parts in the yeast library construction and screen kit (catalogue number 630445) from Clontech. Please find attached the information on these yeast strains as contained with the protocols book for the kit. These yeast form the backbone for the screen for interactors of a library (protein from cDNA from human RNA derived from human ALS-affected or control tissues) to determine what proteins are interacting with a specific protein bait protein. In this screening process, yeast are transformed with vectors carrying coding for protein (or protein fragments) and selected on the basis of viability in medium for which vectors supplement absent amino acids (ie, leucine, histidine), and once interaction between two proteins occurs, yeast are then capable of producing tryptophan, enabling selective growth on tryptophan deficient medium. While we will be preferentially using the AH109, the Y197 strain is also contained in the kit, and thus, will come into our possession.

Description of work involving the HCN-1A human neuronal cells:

These cells are purchased from ATCC, and are immature, self-renewing neuronal cells derived from a megacephalic brain. These cells will be used to determine the interaction between NFL (neurofilament low molecular weight form) and RNA binding proteins (as of yet unknown). We will also be screening for interaction of known proteins (TDP-43 and SOD1) with the NFL mRNA. The reason for purchase is that we needed a human neuronal cell line that would more closely resemble human neurons, so that we can determine RNA binding in a cell specific manner, and, understandably, cannot get human neurons. These cells are not transformed or immortalized, survive to passage 19 only. Please find attached the spec sheet from ATCC for specific details on these cells. A construct containing NFL DNA coding region and another with coding for our proteins of interest will be transfected using lipofectamine into the HCN-1A cells. To determine expression of the NFL mRNA we will be performing several assays: 1: fluorescence assays will determine increased or decreased expression of the EGFP-tagged NFL in the presence of the RNA binding proteins; 2: RT-PCR will determine the level of RNA produced; 3: incubation of cultures with actinomycin will determine the stability of the RNA in the presence of RNA binding proteins; 3: interactions between tau and microtubules by immunohistochemistry/western blotting of protein lysates will be examined; 4: interactions between transfected proteins (SOD1 in particular) and the cell membrane components (immunocytochemistry/western blotting) will also be performed in these cells. For each of these types of experiments these cells will be cultured, transfected, then either fixed or lysed.

Dr. Strong
BIO - RRI - 0035

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr Mike Strong
SIGNATURE (Signature)
DEPARTMENT Cell Biology
ADDRESS Rm 3-15 Robarts
PHONE NUMBER 34452
EMAIL mstrong@uwo.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 3-15
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S)
1) molecular signature of ACS with 2 separate populations
2) Role of macrophia in angiopathy
3) Role of macrophia in infamy

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES (1) ACS Association (USA) (2) Postdoc fund

Names of all personnel working under Principal Investigators supervision in this location

- i) Dr K. Volkenong
- ii) Dr W. Yang
- iii) Dr. M. Gohar
- iv) Wendy Strong
- v) Cheryl Leystra-Lantz
- vi) Katie Moisse
- vii) Samira Humayun

DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? YES NO
 If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen?		Is it known to be an animal pathogen?		Is it known to be a zoonotic agent?		Maximum quantity to be cultured at one time?
	YES/NO	YES/NO	YES/NO	YES/NO	YES/NO		
<i>E. coli</i> - D1152 - XL Blue - 10634	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		100ml				
	<input type="checkbox"/> Yes <input type="checkbox"/> No						
	<input type="checkbox"/> Yes <input type="checkbox"/> No						
	<input type="checkbox"/> Yes <input type="checkbox"/> No						

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required.

① 2 3

1.4 Source of microorganism(s) or biological agent(s)?

In vitro

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 in the table below

Cell Type	Is this cell type used in your work?		Source of Primary Cell Culture Tissue
Human	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		derived from fresh tissue
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No		
Other (specify)			

2.3 Please indicate the type of established cells that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?		Specific cell line(s)	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		EMR32/HEK-293T	ATCC
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		Neuro 2A / PC12 / EOC 20 NSC 391 / BV2 / LADMYC	ATCC
Non-human primate	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			
Other (specify)	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No			

2.4 For above named cell types, circle HC or CFIA containment level required.

① 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 if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids YES NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids YES NO If YES, Specify _____
- Human organs (unpreserved) YES NO If YES, Specify brain
- Human tissues (unpreserved) YES NO If YES, Specify spinal cord

3.3 Is human source known to be infected with and infectious agent YES NO
If YES, please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 (2) 3

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 sequences from the following be involved:

- HIV YES NO
if YES specify _____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens YES NO
if YES specify _____
- Other human or animal pathogen and or their toxins YES NO
if YES specify _____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen YES NO If YES specify SV 40
- Known oncogenes YES NO If YES specify E1A

low OR

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction YES NO
If YES name virus _____

4.5 List specific vector(s) to be used: plasmids

4.6 Will virus be replication defective YES NO

4.7 Will virus be infectious to humans or animals YES NO

4.8 Will this be expected to increase the Containment Level required YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? YES NO

5.3 How will the virus 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 NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? 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 If using murine cell lines, have they been tested for murine pathogens? YES NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used?

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Sheep or goats YES NO
- ◆ Non-Human Primates YES NO If YES specify species _____
- ◆ Wild caught animals YES NO If YES specify species _____ colony # _____

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 _____

8.3 What is the LD₅₀ (specify species) of the toxin _____

9.0 Import Requirements

9.1 Will the agent be imported? YES NO
If no, please proceed to Section 10.0
If yes, country of origin _____

9.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? YES NO

9.4 Has the import permit been sent to OHS? YES NO
If yes, Permit # _____

10.0 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) *Completed*
- ♦ Laboratory and Environmental/Waste Management Safety *→ training will be completed for this last day September*
- ♦ WHMIS) *Completed*

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE: _____
[Handwritten Signature]

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 2 3

11.2 Has the facility been certified by OHS for this level of containment? YES NO

11.3 If yes, please give the date and permit number: Feb. 13, 2006

12.0 Approvals

UWO Biohazard Subcommittee

Signature G.M. Kildner Date 7 Sept, '07

Safety Officer for Institution where experiments will take place

Signature [Handwritten Signature] Date Sept 6/07

Safety Officer for University of Western Ontario (if different than above)

Signature _____ Date _____