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

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: M. Strong

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

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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** *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: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

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	pTRIamp
pGEX-2T	pRSVi
pGEX-4T2	pRK172
pGEX5X-3	pBluescript SK(-)
pAS2-1	pT7T3D
pCR-XL-TOPO	pOTB7
pcII-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 → see email attached
pEYFP-N1	
pEYFP-C1	
pIRES-EGFP	
pBridge	
pEGFP-C1 (new)	
pEGFP-N1	
pECFP-C1	
pSuper	
pShrek-GFP.BAP	
pShrek-hTRA2. BAP	
pShrek-BABP.BAP	

------- 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-RRI-0035

Permit Holder: Mike Strong

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

~~Saima Humayun~~

Katie Moisse

Wendy Strong

May Gohar

Wencheng Yang

Kathy Volkening

Additional Personnel
(Please list additional personnel here)

Jessica Kao

Jen Mepham

	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)	Attlo9 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): Sept 30/08

Chair,Biohazards Subcommittee:

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): J Hanley Sept 30/08

Chair,Biohazards Subcommittee: G.M. Kidder

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 trypophan 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 immunohistochemisty/western blotting of protein lysates will be examined; 4: interactions between transfected proteins (SOD1 in particular) and the cell membrane components (immuncytochemistry/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 x34452
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 ALS with cognitive impairment
2) Role of microglia in tauopathy
3) Role of microglia in injury

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

FUNDING AGENCY/AGENCIES (1) ALS Association (ALSA) (2) Scottish Rite

Names of all personnel working under Principal Investigators supervision in this location:
i) Dr K. Volkening
ii) Dr W. Yang
iii) Dr M. Golvar
iv) Wendy Strong
v) Cheryl Leystra-Lantz
vi) Katie Moisse
vii) Saima 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? 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?
E. coli - DH5α XL1 Blue XL10Gold	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	100ml
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ 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 microrganism(s) or biological agent(s)? Invitrogen

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 (ie. 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	Yes ☒ No	
Rodent	☒ Yes ☐ No	derived from fresh tissue
Non-human primate	☐ Yes ☒ 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	☒ Yes ☐ No	IMR32/HEK-293T	ATCC
Rodent	☒ Yes ☐ No	Neuro 2A / PC12 / EOC 20 NSC34 / BV.2 / LADMAC	ATCC
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

2.4 For above named cell types(s) 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? X 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 X NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES X NO If YES, Specify _____
- Human organs (unpreserved) X YES ☐ NO If YES, Specify brain
- Human tissues (unpreserved) X YES ☐ NO If YES, Specify spinal cord

3.3 Is human source known to be infected with and infectious agent ☐ YES X 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 ? X YES ☐ NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

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

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen X YES ☐ NO If YES specify SV40.
- Known oncogenes X YES ☐ NO If YES specify E1A.

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES X NO
If YES name virus_____.

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

4.6 Will virus be replication defective ☐ YES X NO

4.7 Will virus be infectious to humans or animals ☐ YES X NO

4.8 Will this be expected to increase the Containment Level required ☐ YES X 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 LD_{50} (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 _____

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. Fidler Date 7 Sept. '07

Safety Officer for Institution where experiments will take place

Signature [illegible] Date Sept 6/07

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

Signature _____ Date _____

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