Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

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

Jessica Kao

Cheryl Leystra-Lantz

Wendy Strong

May Gohar

Wencheng Yang

Kathy Volkening

Cristian Droppelmann

Additional Personnel
(Please list additional personnel here)

Zhong Ping He
Brian Keller
Danae Campos-Melo

	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), 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	pmir GLO pGL3-Control
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.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: 

Classification: 2

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

Date of Last Modification (if applicable): Sep 9, 2009

BioSafety Officer(s): Ronald Worsley 27/04/10

Chair, Biohazards Subcommittee:

The new vectors, pmirGLO and pGL3-Control, are to be used for luciferase assays.

Vectors	Location	Notes	Supplier	
pCMX	F2-S3-R1-A: A1	mammalian expression		
pGEX-4T2	F2-S3-R1-A: A2	GST-fusion/thrombin cleavage		
pGEX5X-3	F2-S3-R1-C: E1,2	GST-fusion/factor Xa cleavage		
pGAD-424	F2-S3-R1-A: A3	yeast two-hybrid screen AD & Leu screen		
pAS2-1	F2-S3-R1-A: A4	yeast two-hybrid screen BD		
pcDNA3.1(+)	F2-S3-R1-B: I8	mammalian expression		
	F2-S3-R1-D: E1-3			
pcDNA3.1-myc hisA	F2-S3-R1-A: A5			
pcDNA3.1-myc hisB	F2-S3-R1-A: A6			
pcDNA3.1-myc hisC	F2-S3-R1-A: A7			
pRFP-N1	F2-S3-R1-A: A8	red fluorescent protein @583nm		
pRFP-N2	F2-S3-R1-A: B1	red fluorescent protein @583nm		
pRFP-N3	F2-S3-R1-A: C1			
pGAD-T7	F2-S3-R1-A: B2	shuttle vector- E.coli↔yeast + HA tag	Clontech	
pEYFP-N1	F2-S3-R1-A: B3	yellow fluorescent protein @527nm		
pEYFP-C1	F2-S3-R1-A: B4	yellow fluorescent protein @527nm		
pIRES-EGFP	F2-S3-R1-A: B5			
pBridge	F2-S3-R1-A: B6	yeast, two MCS's		
pEGFP-C1 (new)	F2-S3-R1-A: B7, B8	green fluorescent protein @507nm		
pEGFP-N1	F2-S3-R1-C:D1			
pECFP-C1	F2-S3-R1-A: C8,9	cyan fluorescent protein @475nm		
pcDNA/CMV-HA ubiquitin	F2-S3-R1-A: C3			
pSuper	F2-S3-R1-A: F1			
pShrek-GFP.BAP	F2-S3-R1-A: E1			
pShrek-hTRA2. BAP	F2-S3-R1-A: E2			
pShrek-BABP.BAP	F2-S3-R1-A: E3			
pcDNA-BrA	F2-S3-R1-A: E4			
pBluescript SK(-)	F2-S3-R1-C: C6,7			
pmirGLO		Dual-Luciferase miRNA Target Expression	Promega	NEW
pGL3-Control			Promega	NEW

Certificate of Analysis

pmirGLO Dual-Luciferase miRNA Target Expression Vector:

Cat.#	Size
E1330	20µg

Part# 9PIE133
Revised 10/09

Cat.# E1330 contains:

Part No.	Name	
E133A	pmirGLO Vector	20µg
C838A	Oligo Annealing Buffer	1ml

Description: The pmirGLO Dual-Luciferase miRNA Target Expression Vector[a-e] is designed to quantitatively evaluate microRNA (miRNA) activity by the insertion of miRNA target sites 3´ of the firefly luciferase gene (*luc2*). These target sites can be introduced by cloning putative miRNA binding sites alone, or the 3´ untranslated region (UTR) of a gene of interest, to study the influence of these sites on transcript stability and activity. Firefly luciferase is the primary reporter gene; reduced firefly luciferase expression indicates the binding of endogenous or introduced miRNAs to the cloned miRNA target sequence. This vector is based on Promega dual-luciferase technology, with firefly luciferase (*luc2*) used as the primary reporter to monitor mRNA regulation and *Renilla* luciferase (*hRluc-neo*) acting as a control reporter for normalization and selection. This vector contains the following features:

- Human phosphoglycerate kinase (PGK) promoter provides low translational expression, which is advantageous when reduction of signal is the desired response. The PGK promoter is a nonviral universal promoter, which functions across cell lines (yeast, rat, mouse and human).
- Firefly luciferase reporter gene (*luc2*) inversely reports miRNA activity in mammalian cells.
- Multiple cloning site (MCS) is located 3´ of the firefly luciferase reporter gene (*luc2*).
- Humanized *Renilla* luciferase-neomycin resistance cassette (*hRluc*-neo) is used as a control reporter for normalization of gene expression and stable cell line selection.
- Amp^r gene allows bacterial selection for vector amplification.
- SV40 late poly(A) signal sequence is positioned downstream of *luc2* to provide efficient transcription termination and mRNA polyadenylation.
- Synthetic poly(A) signal/transcription stop site.

Concentration: 1µg/µl in 10mM Tris-HCl, 1mM EDTA; final pH 7.4.

GenBank® Accession Number: FJ376737.

Storage Conditions: See the storage temperature and expiration date on the Product Information Label.

Quality Control Assays

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: **www.promega.com/vectors/**

Restriction Digestion: The functional purity of this vector DNA is verified by complete digestion with restriction enzymes at the optimal temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of this vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Signed by: J. Stevens

J. Stevens, Quality Assurance

Promega

Promega Corporation

2800 Woods Hollow Road	
Madison, WI 53711-5399	USA
Telephone	608-274-4330
Toll Free	800-356-9526
Fax	608-277-2516
Internet	www.promega.com

Part# 9PIE133
Printed in USA Revised 10/09

[a]READ THIS FIRST BEFORE OPENING PRODUCT
The sale of this product and its use by the purchaser are subject to the terms of a limited use label license, the full text of which is shipped with this product and also available at: **www.promega.com/LULL**. That text must be read by the purchaser prior to opening this product to determine whether the purchaser agrees that all use of the product shall be in accordance with the license terms. If the purchaser is not willing to accept the terms of the limited use label license, Promega is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, if the product is opened for any reason, then the purchaser agrees to be bound by the terms of the limited use label license.
[b]U.S. Pat. No. 5,670,356.
[c]Australian Pat. No. 2001 285278 and other patents pending.
[d]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.
[e]Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

Promega

Features List and Map for the pmirGLO Vector

SV40 late poly(A) signal	106–327
SV40 early enhancer/promotor	426–844
hRluc-neo fusion protein coding region	889–2664
Synthetic polyadenylation signal	2728–2776
β-lactamase (Amp^r) coding region	3037–3897
*Col*E1-derived plasmid origin of replication	4052–4088
Human phosphoglycerate kinase promoter	5094–5609
luc2 reporter gene	5645–7297
Multiple cloning site (MCS, Figure 1)	7306–7350

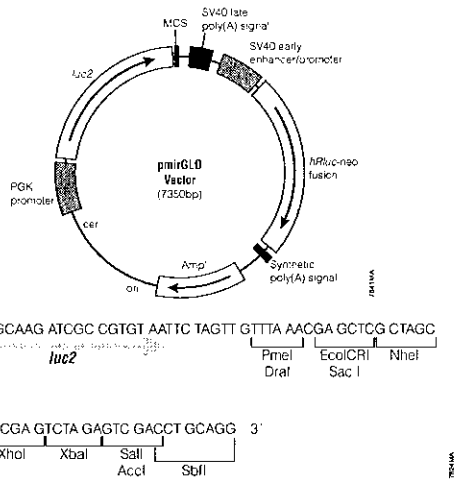


Figure 1. pmirGLO Vector multiple cloning site.

I. Sample Protocol

A. Vector Cloning

1. Design oligonucleotides: Order oligonucleotide pairs that contain the desired miRNA target region and, when annealed and ligated into the pmirGLO Vector, result in the miRNA target region in the correct 5′ to 3′ orientation. Insure that the overhangs created by oligonucleotide annealing are complementary to those generated by restriction enzyme digestion of the pmirGLO Vector in Step 2. Add an internal restriction site to your oligonucleotides for clone confirmation (e.g., NotI in Figure 3 creates a ~125bp insert when digested with NotI because of a NotI site at position 93 in the vector).
2. Digest vector: Linearize the pmirGLO Vector with the appropriate restriction enzymes to generate overhangs that are complementary to the annealed oligonucleotide overhangs.
3. Anneal oligonucleotides: Dilute both oligonucleotides (supplied by user) to 1µg/µl. Combine 2µl of each oligonucleotide with 46µl of Oligo Annealing Buffer. Heat at 90°C for 3 minutes, then transfer to a 37°C water bath for 15 minutes. Use the annealed oligonucleotides immediately, or store at –20°C.

B. Ligation and Transformation

1. Dilute annealed oligonucleotides 1:10 in nuclease-free water to a final concentration of 4ng/µl per oligonucleotide. Ligate 4ng of annealed oligonucleotides and 50ng of linearized vector using a standard ligation protocol. Transform ligated pmirGLO Vector using high-efficiency JM109 competent cells (e.g., Cat.# L2001).
2. Select clones on ampicillin-containing plates, then select clones containing the oligonucleotides by digesting miniprep-purified DNA (e.g., purified using the PureYield™ Plasmid Miniprep System, Cat.# A1221) using the unique restriction site in the oligonucleotide pair. The purified plasmid DNA can be transfected directly or expanded to generate more DNA.

Additional information about annealing, ligation, transformation and oligonucleotide design can be found in the *GeneClip™ U1 Hairpin Cloning Systems Technical Manual*, #TM256, which is available at: **www.promega.com/tbs/**

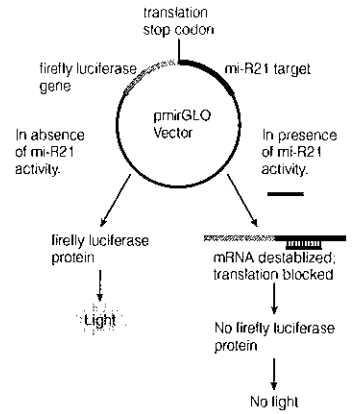


Figure 2. Mechanism of action of the pmirGLO Vector.

	PmeI NotI internal site	miR21 target sequence	XbaI
miR21 sense, PmeI and XbaI	5′ **AAAC** TA GCGGCCGC TAGT TCAACATCAG *TCT*GATAAGCTA T 3′		
miR21 mismatch sense, PmeI and XbaI	5′ **AAAC** TA GCGGCCGC TAGT TCAACATCAG *AA*GATAAGCTA T 3′		

	XbaI	miR21 target sequence	NotI internal site	PmeI
miR21 antisense, PmeI and XbaI	5′ **CTAGA** TAGCTTATCAGACTGATGTTGA ACTA GCGGCCGC TA **GTTT** 3′			
miR21 mismatch antisense, PmeI and XbaI	5′ **CTAGA** TAGCTTATC*TT*CTGATGTTGA ACTA GCGGCCGC TA **GTTT** 3′			

Figure 3. Sample oligonucleotides for miR21.

C. An Example of Detecting miR21 Activity Using the pmirGLO Vector:miR21 Construct

An overview describing the use of the pmirGLO Vector to interrogate endogenous miR21 microRNA is shown in Figure 2.

The presence of broadly endogenous microRNA miR21 was monitored in HeLa cells. Constructs contained either an exact match to the 21bp miR21 target sequence or a mismatched version of that target site (1) as well as PmeI, XbaI and NotI restriction sites (Figure 3; mismatched sequence is in italics). Twenty-four hours after transfection with the miR21 pmirGLO Vector constructs, cells were analyzed for luciferase activity using the Dual-Glo® Luciferase Assay System (Cat.# E2920) and a MicroLumatPlus LB96V luminometer (Berthold). Normalized firefly luciferase activity (firefly luciferase activity/*Renilla* luciferase activity) for each construct was compared to that of the pmirGLO Vector no-insert control. For each transfection, luciferase activity was averaged from six replicates.

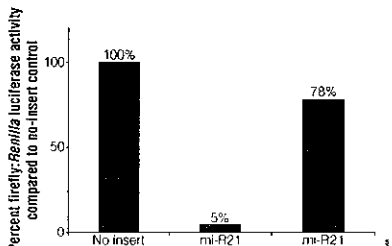


Figure 4. Normalized luciferase activity using the pmirGLO Vector with an miR21 target sequence.

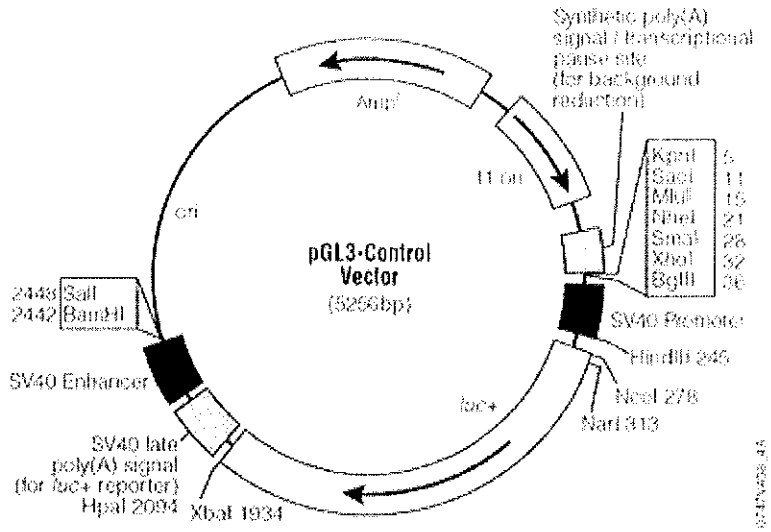
II. Reference

1. Zeng, Y. and Cullen, B.R. (2003) Sequence requirements for micro RNA processing and function in human cells. *RNA* **9**, 112–23.

Part# 9PIE133
Printed in USA Revised 10/09

pgl3-control vector

e1741



Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

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)

Zhang Ping He

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Classification: 2

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

Signature of Permit Holder Aug 26 09

BioSafety Officer(s): Sept 9/09

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), Alt109 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	
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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 see page 1

BioSafety Officer(s): J'Stanley Sept 9/09

Chair,Biohazards Subcommittee: C.M. Kidder

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

invitrogen

Material Safety Data Sheet

Revision Date: 24-Mar-2008

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/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

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/m³ (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 PARTCULAR PURPOSE.

End of Safety Data Sheet

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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

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Approved Use of Human Source Material	brain, spinal cord	
Approved GMO	SV 40 Large T antigen, E1A	

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Classification: 2

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

Signature of Permit Holder: [signature] June 26/09

BioSafety Officer(s): [signature]

Chair, Biohazards Subcommittee: [signature]

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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Signature of Permit Holder: See page 1.

BioSafety Officer(s): V Stanley June 20/07

Chair,Biohazards Subcommittee: E K. Kolder

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
peII-TOPO	pGEM-Teasy
peDNA3.1(+)	pGEM4Z
peDNA-BrA	pGEM7Zf
peDNA3.1-myc hisA	pGBKT7-53
peDNA3.1-myc hisB	pGBKT7-LAM
peDNA3.1-myc hisC	pGBKT7-DNA-BD
peDNA/CMV-HA ubiquitin	pCMV-SPORT6
pRFP-N1	pGAD-424
pRFP-N2	pGAD-T7
pRFP-N3	pAG306Gal-ccdB-EGFP -> see e'mail 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

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Katie Moisse	Jen Mepham
Wendy Strong	
May Gohar	
Wencheng Yang	
Kathy Volkening	

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Approved GMO	SV 40 Large T antigen, E1A	

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Date of last Biohazardous Agents Registry Form Sep 7, 2007

Signature of Permit Holder:

BioSafety Officer(s): [signature] dept 30/08

Chair,Biohazards Subcommittee: [signature]

Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

Approved use of Animals

Approved Toxin(s)

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Signature of Permit Holder:

BioSafety Officer(s): 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 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 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: [illegible]
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) [illegible]
2) Role of microglia [illegible]
3) Role of microglia [illegible]

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

[illegible]

[illegible]
i Dr K Volkening
Dr W Yang
Dr M. Gohar
Wendy Strong
Cheryl Leystra-Lantz
vii Katie Moisse
viii Saima Humayun

[illegible]

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)? X 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 - XL Blue MRF'	☐ Yes X No	☐ Yes X No	☐ Yes X No	100 mL
	☐ 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 microorganism(s) or biological agent(s)? Invitrogen

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X YES ☐ NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (ie. derived from fresh tissues) 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 X No	
Rodent	X Yes ☐ No	derived from fresh tissue
Non-human primate	☐ Yes X 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
	X Yes ☐ No	EMR32/HEK-293T	ATCC
	X Yes ☐ No	Neuro 2A / HeLa / SOC 18 NSC 34 / BV2 / ARPMC	ATCC
	☐ Yes X No		
	☐ Yes X No		

XO V

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 4 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 ✓ YES NO If YES specify SV40
- Known oncogenes ✓ YES NO If YES specify EIA

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

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

4.6 Will vectors have replication deficient ○ YES X NO

4.7 Will vectors be infectious to humans or animals ○ YES X NO

4.8 Will [illegible] ○ YES X [illegible]

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 [illegible]

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

UTTO Biohazard Subcommittee

Signature G.M. Kirkham Date 7 Sept, 07

Safety Officer for Institution where Experiment is to Take Place

Signature D. Fowler Date Sept 6/07