Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

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

Jamie Simek
Cindy Pan
~~Katharine Toth~~
~~Steve Celetti~~
Ruchi Bhalla
Jared Churko
~~Kyle Cowan~~
Silvia Peneula
Stephanie Langlois
Isabelle Plante
Xiang-Qun Gong
Cindy Shao
~~Jennifer Siu~~ leave on list D.T.

Additional Personnel
(Please list additional personnel here)

JACK LEE
ROBERT LORENTZ
JASON MARTIN

* *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: Mar 27, 2009

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-0017

Permit Holder: Dale Laird

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. coli DH5 alpha, JM109	
Approved Cells	Human (primary), skin biopsies, Rodent (primary), transgenic alpha mutant mouse, Human (established), HELA, 293T, 293 Hek, Tumour cell lines, Rodent (established), Keratinocytes, NRK BiCR, MIRK, N2A,	B16-F0 B16-F10
Approved Use of Human Source Material	Human blood (whole), Human tissues (unpreserved), Human tissues (preserved), ODDD Patients and relatives	
Approved GMO	JM109, T-EASY, PcDNA3, pEGFP, retrovirus AP-2, SV 40 Large T antigen, HEK, E1A oncogenes, HeLa	PGFP-V-RS VECTOR
Approved use of Animals	mice	
Approved Toxin(s)		

New cells to be used for connexin and pannexin studies. New vectors to be used to silence Panx 1. Additional Biohazards are level 1.

* *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: Mar 27, 2009

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

HuSH shRNA Plasmid, pGFP-V-RS

pGFP-V-RS shRNA Cloning Plasmid
Catalog # TR30007

Product Description:

- Plasmid vector for cloning shRNA expression cassettes
- Designed for long term gene silencing studies
- Kanamycin (25ug/ml) and Puromycin resistance markers for easy selection of transformed or transfected cells
- U6 polymerase III promoter for shRNA expression
- MMLV LTR sequences for packaging into retroviral particles
- EcoRI and HindIII sites convenient for shuttling existing HuSH cassettes

Content: Each vial contains 5 ug of dried and purified plasmid DNA.

Storage and Stability: The plasmid is stable for at least 1 yr at –20°C from the date of shipment.

Guarantee: This product is guaranteed for the correct sequences and listed functions.

Related Products: Specific HuSH constructs are available at OriGene covering the full human, mouse and rat genomes.

Quality Control Assays

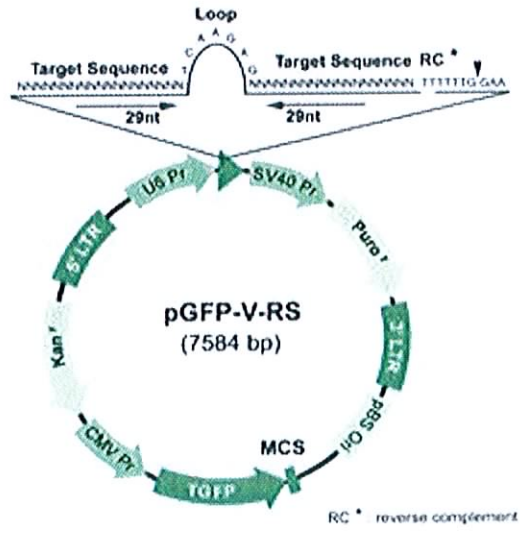
DNA Quantitation: The concentration of the purified plasmid was determined at OD_{260} by a UV spectrometer.

DNA Sequence Analysis: The final purified plasmid was sequenced to confirm its identity.

Functional Analysis:

1. Cloning: the pGFP-V-RS plasmid was digested with BamHI and HindIII and the digested fragment isolated. Multiple shRNA expression cassettes were cloned into this plasmid.
2. Inhibition of target gene: shRNA constructs cloned into pGFP-V-RS were verified for inhibition of target genes.
3. Stable cell lines: pGFP-V-RS was verified to generate stable cell lines using direct transfection.

Figure 1: Map of shRNA Cloning Vector pGFP-V-RS



Terms of Use

By opening the use the product, the purchaser agrees not to distribute, resell, modify for resale or use to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree with these conditions, please return product to OriGene for a full refund.

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item **Price:** $264.00

Designations: B16-F10

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: melanocyte

PHOTO

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Applications: transfection host (technology from amaxa)

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

Subculturing:

Related Links

- NCBI Entrez Search
- Cell Micrograph
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790

22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987

22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res. 37: 3945-3956, 1977. PubMed: 908034

22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895, 1977. PubMed: 887927

23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. J. Natl. Cancer Inst. 60: 1217-1222, 1978. PubMed: 418183

23362: . . Nat. New Biol. 242: 148-149, 1973.

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: CRL-6322™ Order this Item **Price:** $318.00

Designations: B16-F0

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: Spindle shaped

Source:
Organ: skin
Strain: C57BL/6J
Disease: melanoma

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Applications: transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)

Tumorigenic: Yes

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:

Related Links
- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:10 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:
Freeze medium: Complete growth medium 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22151: Fidler IJ. Biological behavior of malignant melanoma cells correlated to their survival in vivo. Cancer Res. 35: 218-224, 1975. PubMed: 1109790
22191: Fidler IJ, et al. Tumoricidal properties of mouse macrophages activated with mediators from rat lymphocytes stimulated with concanavalin A. Cancer Res. 36: 3608-3615, 1976. PubMed: 953987
22192: Fidler IJ, Bucana C. Mechanism of tumor cell resistance to lysis by syngeneic lymphocytes. Cancer Res. 37: 3945-3956, 1977. PubMed: 908034
22243: Fidler IJ, Kripke ML. Metastasis results from preexisting variant cells within a malignant tumor. Science 197: 893-895, 1977. PubMed: 887927
22424: Fidler IJ. Immune stimulation-inhibition of experimental cancer metastasis. Cancer Res. 34: 491-498, 1974. PubMed: 4812256
23224: Briles EB, Kornfeld S. Isolation and metastatic properties of detachment variants of B16 melanoma cells. J. Natl. Cancer Inst. 60: 1217-1222, 1978. PubMed: 418183
23362: . . Nat. New Biol. 242: 148-149, 1973.

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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 2008
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be established 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, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR DALE W. LAIRD
SIGNATURE
DEPARTMENT ANATOMY & CELL BIOLOGY
ADDRESS DSB 00077
PHONE NUMBER x 86827
EMAIL DALE.LAIRD@SCHULICH.UWO.CA

Location of experimental work to be carried out: Building(s) DSB Room(s) 00076 / 00070

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR
GRANT TITLE(S): CX43 MUTATIONS LINKED TO HUMAN DISEASE

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

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

DR. QING (CINDY) SHAO
DR. STEPHANIE LANGLOIS
DR. SILVIA PENUELA
DR. ISABELLE PLANT
DR. GREGORY GOING
JARED CHURKO
RUCHI BHALLA
STEVE CELETTI
KATHERINE TOTH
JENNIFER SIU
JAMIE SIMEK

1.0 Microorganisms

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

GRANT RENEWED APRIL 2009

Cx43 mutations linked to human disease

Intercellular gap junction channels allowing the direct passage of small molecules and secondary messengers between most contacting cells are built from a library of 21 connexin (Cx) family members. At present, 8 distinct genetic diseases ranging from sensorineural deafness to developmental disorders have been linked to germ line mutations in the genes encoding connexins. To date, 42 mutations in the *GJA1* gene encoding the gap junction protein, Cx43 have been linked to the human developmental disorder known as oculodentodigital dysplasia (ODDD). This primarily autosomal dominant disease is typically characterized by syndactyly, camptodactyly, craniofacial abnormalities, enamel loss, incontinence and ophthalmic defects. Given that Cx43 is the predominant connexin expressed in over 35 distinct cell types, it is remarkable that patients have moderate to severe defects in some organs while other organs appear to remain free of developmental abnormalities and disease. Although many mutants are distributed on the cell surface not unlike wild-type Cx43, all mutants tested to date exhibit complete or substantial loss of function and dominant-negative effects on coexpressed wild-type Cx43 with respect to gap junctional intercellular communication (GJIC). Mechanistically, at least some of these mutants appear to act as dominant-negatives by direct co-oligomerization with wild-type Cx43, although their effects *in vitro* and *in vivo* on other coexpressed connexins remain unknown. Thus, we **hypothesize that different ODDD-linked Cx43 mutants exhibit distinct cellular phenotypes and effects on coexpressed connexins manifesting in a loss of GJIC and perturbed cell differentiation, ultimately resulting in variable disease load.**

Aim 1: Examine the functional status, fate, dynamics and inter-connexin interactions of dominant and recessive ODDD-linked Cx43 mutants in reference cells and in cells obtained from ODDD patients. Dominant and recessive Cx43 mutants will be characterized with respect to (1) the ability to exert dominant and transdominant effects on GJIC and (2) channel function. These parameters will be assessed in defined reference cell models and primary cell cultures from ODDD-linked mutant mice that coexpress Cx43 or other connexins typically coexpressed with Cx43 *in vivo*. FRET, co-immunoprecipitation and pulse-chase studies will be used to determine if the mutants establish direct interactions with coexpressed connexins and regulate their functional half-life. Finally, the expression, localization, phosphorylation status, and turnover of Cx43 will be examined in fibroblasts and/or tissue biopsies obtained from a cohort of patients harbouring Cx43 gene mutations.

Aim 2: *Characterize transgenic mouse models of ODDD that harbor missense or truncation mutations in distinct regions of the Gja1 gene and analyze the consequences of the mutants on cell differentiation.* Interestingly, current mouse models harboring different ODDD-linked mutants exhibit both similar and distinct phenotypes that echo the diversity of symptoms observed in ODDD patients. Thus, we will first finish the generation and characterization of a "knock-in" gene-targeted mouse in which the endogenous Cx43 coding sequence is replaced either by the human wild-type Cx43 coding sequence or the Cx43[illegible] mutant coding sequence. Together with the $Cx43^{G60S}$, $Cx43^{I130T}$, $Cx43^{G138R}$ and littermate control mice, we will compare the phenotypes of these mutant mouse models of human ODDD by performing *in situ* analysis using combinations of microCT and microMRI analysis, in addition to immunohistochemistry for connexins and tissue specific differentiation markers. Cell differentiation and function will be further assessed in primary cell cultures and/or explants from mineralized tissue (osteoblasts and chondroblasts), where abnormalities are consistently prevalent in patients, smooth muscle cells and myofibroblasts from the bladder, as approximately 30% of the patient cohort report urinary problems and the mammary gland (myoepithelial and luminal cells), where subclinical disease may be present. Lastly, BrdU incorporation and Ki67 immunolabeling as well as TUNEL and caspase 3 staining will be used to assess the effect of Cx43 mutants on cell proliferation and cell death, respectively.

These studies will combine reference cell models expressing ODDD mutants, mouse models of ODDD and direct ODDD patient data to establish genotype-phenotype relationships and the role of Cx43 in cell differentiation and function.

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 O NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ☒ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	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	Health Canada or CFIA Containment Level
DH5 α E coli	O Yes ☒ No	O Yes ☒ No	O Yes ☒ No	1 500 ML	INVITROGEN	☒ 1 O 2 O 3
JM109	O Yes ☒ No	O Yes ☒ No	O Yes ☒ No	1 500 ML	PROMEGA	☒ 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 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? ☒ YES O 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	AUS Protocol Number
Human	☒ Yes O No	SKIN BIOPSIES	Not applicable
Rodent	☒ Yes O No	TRANSGENIC & MUTANT MICE	2006 - 101
Non-human primate	O Yes ☒ No		
Other (specify)	O Yes ☒ No		

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	HELA, 293T, 293 HEK, TUMOR CELL LINES	ATCC
Rodent	☒ Yes ○ No	KERATINOCYTES, NRK DICR-MIB$_K$, N2A	CLONETIC, ATCC LINES, INHOUSE CELL
Non-human primate	○ Yes ☒ No		
Other (specify)	☒ Yes ○ No	MOCK	ATCC

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) THESE ARE ALL STANDARD WELL USED CELL LINES

2.4 For above named cell types(s) indicate 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 in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Organs or Tissues (preserved)	ODDD PATIENTS AND RELATIVES	○ Yes ☒ No		☒ 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 modification(s) involving plasmids be done? ☒ YES, complete table below ○ NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
SM109	T-EASY PC DNA3 (+)(-) PEGFP	PROMEGA INVITROGEN CLONETECH	CONNEXIN GENES PANNEXIN GENES	CELL LINES EXPRESSING PLASMIDS TEND TO GROW SLOWER AND FORM CELL-CELL OR CELL CHANNELS.

* Please attach a Material Data Sheet or equivalent if available.

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

4.3 Will genetic modification(s) involving viral vectors be done? ☒ YES, complete table below ○ NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
RETROVIRUS	AP-2	DR. J. GALIPEAU McGILL UNIVERSITY	CONNEXINS PANNEXINS	CELLS COMMUNICATE BETTER

* Please attach a Material Safety Data Sheet or equivalent. SEE BIO-UWO-0017 AS INFORMATION IS CURRENTLY ON RECORD WITH SAFETY OFFICE

4.4 Will genetic sequences from the following be involved?

- HIV ○ YES, please specify _____ ☒ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ○ YES, specify _____ ☒ NO
- SV 40 Large T antigen ○ YES {HEK cells ☒ NO ?
- E1A oncogene ○ YES {HeLa cells ☒ NO ?
- Known oncogenes ○ YES, please specify _____ ☒ NO
- Other human or animal pathogen and or their toxins ○ YES, please specify _____ ☒ NO

4.5 Will virus be replication defective? ☒ YES ○ NO

4.6 Will virus be infectious to humans or animals? ☒ YES ○ NO

4.7 Will this be expected to increase the containment level required? ○ YES ☒ NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? ○ 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, number: _____ ○ NO ○ PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ☒ YES ○ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used MICE

6.3 AUS protocol # 2006-101

6.4 Will any of the agents listed be used in live animals ○ YES, specify: _____ ☒ 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 body fluids including blood be used?

- Pound source dogs O YES ☒ NO
- Pound source cats O YES ☒ NO
- Cattle, sheep or goats O YES ☒ NO
- Non- Human Primates O YES, please specify species _____ ☒ NO
- Wild caught animals O YES, please specify species & colony # _____ ☒ NO
- Birds O YES ☒ NO
- Others (wild or domestic) O YES, please specify _____ ☒ NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ☒ NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☒ NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

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

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please 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_____
If no, please proceed to Section 10.0 ⊘NO

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
X YES, permit # if on-campus BIO - UWO - 0017
O NO
O NOT REQUIRED

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: March 4, 09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 31 March 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: March 27/09

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: BIO-UWO-0017 Expiry Date (3 years from Approval): March 26, 2012

Special Conditions of Approval:

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

-------- Original Message --------
Subject:questions
Date: Tue, 03 May 2005 09:17:07 -0400
From: Dale Laird <Dale.Laird@fmd.uwo.ca>
To:jstanle2@uwo.ca
CC: Cindy Shao <Cindy.Shao@fmd.uwo.ca>

Dear Jennifer, I apologize for the delay in responding to your questions and comments on my last Biohazardous Agents Registry Form. Your email was sent to dwlaird@uwo.ca instead of dale.laird@fmd.uwo.ca and got held back for some reason. I found 650 emails on my UWO account of which many were not forwarded to my FMD account (as they should be). Anyway here is the additional information you requested.

1. Section 4.3 - please note that HEK 293 contains E1A oncogene
Response: Thank you this is noted.

2. Section 4.5 -

Response: -The AP-2 retroviral vector is the main one we have been using for several years. The documentation, vector source and description was placed on file with the Safey office several years ago. See manuscript reference Galipeau et al., 1999, Cancer Research 59: 2384-2394. The 293GPG packaging cells which produce replication-defective virus are described in this same paper. The 293GPG packaging cells were orginally described in Ory et al., Proc Natl Acad Sci U S A. 1996 Oct 15;93(21):11400-6. Both the AP-2 vector and packaging cells are from Dr. Jacques Galipeau in Montreal. The MTA for these cells and vectors were done in collaboration with Dr. Chris Naus several years ago.

-More recently we have obtained thepH1.1-QCXIH retroviral vector (from GenScript) for shRNA studies. See (Barton and Medzhitov, 2002, PNAS 99; 14943-14945). The HEK293 derived packaging cells produce replication-incompetent viral particles. (See AmphoPack293 from BD Biosciences).
3: Section 1.2 - confirm E. coli DH5alpha,
Response: -Yes this is the E.coli we use,

4: Section 6.0 - do you have an animal protocol?
Response: -The animal studies being performed in this study are in conjunction with the Co-Principle Applicants, Drs. Kidder and Dr. Bernier. Their animal protocols have been modified to include this new grant. I do not have a separate animal protocol.

5: Section 8.3 - description of lindane use:
Response: -Lindane is disolved in DMSO at 50um and used as a gap junction channel inhibitor at a final concentration of 50nM.

I trust this answers all your questions. Again sorry for the delay.

Dale Laird

Dale W. Laird, Ph.D.
Professor
Canada Research Chair in Gap Junctions and Disease