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

Ruchi Bhalla

Jared Churko

Silvia Peneula

~~Stephanie Langlois~~

Isabelle Plante

Xiang-Qun Gong

Cindy Shao

Jason Martin

Robert Lorentz

Jack Lee

Jennifer Siu

Additional Personnel
(Please list additional personnel here)

PASQUALE VECCHIO

	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,	C2C12, L6, L10BIOBR - GFP L10BIOBR - MAPKK
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	
Approved use of Animals	mice	

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: Mar 27, 2009

Date of Last Modification (if applicable):

BioSafety Officer(s):

Chair, Biohazards Subcommittee:

See attached sheets

The CRL-2770 and CRL2771 are mouse melanoma cell lines. We are interested in investigating the role of pannexins and connexins as tumor suppressors in these cells. In the event the cells have sufficient pannexins and/or connexins we will knockdown their expression by RNAi technologies. If they are low in pannexins and/or connexins we will overexpress these molecules and examine the effect of these molecules on cell characteristics associated with cell migration and invasion. In the event that one or more pannexins/connexins affects their growth characteristics we will attempt to determine the mechanism involved.

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Cell Biology

ATCC® Number: CRL-2771™ Order this Item
Price: $338.00

Designations: L10BIOBR-MAPKK
Depositors: JL Arbiser
Biosafety Level: 2 [Cells containing SV40 viral DNA sequences]
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)
Morphology: melanocyte
PHOTO

Source: **Cell Type:** melanocyte;
Strain: B10.BR

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.

Isolation: **Isolation date:** January 1, 2002

Applications: tumor model

Tumorigenic: Yes

Age: newborn

Comments: The L10BIOBR-MAPKK cell line (ATCC CRL-2771) was derived by infecting the immortalized murine melanocyte cell line, L10BIOBR, with pBABE which encodes a constitutively active MAPKK. The vector contains the SV40 viral DNA sequences and the puromycin resistance gene. The cells were selected in medium containing puromycin.The introduction of the MAPKK gene into melanocytes leads to tumorigenesis in nude mice, activation of the angiogenic switch and increased production of the proangiogenic factor, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMPs). Activation of MAP kinase signaling may be an important pathway involved in melanoma transformation. Inhibition of MAP kinase signaling may be useful in the prevention and treatment of melanoma. The L10BIOBR-MAPKK cell line and the corresponding negative control, L10BIOBR-GFP (CRL-2770), are a model for melanoma tumorigenesis and signal transduction [PubMed: 12514183].

Propagation: **ATCC complete growth medium:** Ham's F10 medium supplemented with 50 ng/ml TPA (Sigma Catalogue No. P-8139) and 7% horse serum
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

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Subculturing:	**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. An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/sq. cm. is recommended. 6. Incubate cultures at 37°C. **Interval:** Subculture when cells reach a concentration of 4 X 10(4) cells/sq. cm. **Subcultivation Ratio:** A subcultivation of 1:6 to 1:8 is recommended **Medium Renewal:** Two to three times weekly
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	24 hours
Related Products:	recommended serum:ATCC 30-2040 derived from same cell line:ATCC CRL-2770
References:	89472: Govindarajan B, et al. Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling. J. Biol. Chem. 278: 9790-9795, 2003. PubMed: 12514183

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Cell Biology

ATCC® Number: CRL-1458™ Order this Item
Price: $264.00

Designations: L6
Depositors: D Schubert
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: Rattus norvegicus (rat)
Morphology: myoblast

Source: **Tissue:** skeletal muscle
Cell Type: myoblast myoblast;
Cellular Products: myosin

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Comments: The L6 myogenic line was isolated originally by Yaffe from primary cultures of rat thigh muscle maintained for the first two passages in the presence of methyl cholanthrene. [22581]
L6 cells fuse in culture to form multinucleated myotubes and striated fibers. The extent of cell fusion declines with passage and the cells should be frozen at low passage and periodically recloned with selection for fusion competent cells.
Tested and found negative for ectromelia virus (mousepox).

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
Growth Conditions: The myoblastic component of this line will be depleted rapidly if the cells are allowed to become confluent.

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Subculturing:

Protocol: Subculture before the cells become confluent to retard the loss of differentiating ability that is observed as the cells are passaged.

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 1:20 to 1:40 is recommended
Medium Renewal: 2 to 3 times per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
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:

1064: Mandel JL, Pearson ML. Insulin stimulates myogenesis in a rat myoblast line. Nature 251: 618-620, 1974. PubMed: 4421831
22255: Richler C, Yaffe D. The in vitro cultivation and differentiation capacities of myogenic cell lines. Dev. Biol. 23: 1-22, 1970. PubMed: 5481965
22581: Yaffe D. Retention of differentiation potentialities during prolonged cultivation of myogenic cells. Proc. Natl. Acad. Sci. USA 61: 477-483, 1968. PubMed: 5245982
33164: Osawa H, et al. Identification and characterization of basal and cyclic AMP response elements in the promoter of the rat hexokinase II gene. J. Biol. Chem. 271: 17296-17303, 1996. PubMed: 8663388
33165: Osawa H, et al. Analysis of the signaling pathway involved in the regulation of hexokinase II gene transcription by insulin. J. Biol. Chem. 271: 16690-16694, 1996. PubMed: 8663315

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Cell Biology

ATCC® Number: CRL-2770™ Order this Item
Price: $338.00

Designations: L10BIOBR-GFP
Depositors: JL Arbiser
Biosafety Level: 1
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Mus musculus* (mouse)
Morphology: melanocyte
PHOTO
Source: **Cell Type:** melanocyte;
Strain: B10.BR
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.
Isolation: **Isolation date:** January 1, 2002
Tumorigenic: No
Age: newborn
Comments: The L10BIOBR-GFP cell line was derived as a negative control for CRL-2771. The immortalized murine melanocyte cell line L10BIOBR was transduced with pDIVA-GFP and subjected to puromycin selection. Together, L10BIOBR-GFP (CRL-2770) and L10BIOBR-MAPKK (ATCC ® CRL -2771) are valuable cell models for oncogenic transformation and signal transduction studies for melanoma [PubMed: 12514183]. **Please note,** although the L10BIOBR-GFP cell line harbors the gfp gene, as verified by PCR analysis, the cell line does not express sufficient GFP protein for detection of GFP fluorescence by flow cytometry or fluorescence microscopy.
Propagation: **ATCC complete growth medium:** Ham's F10 medium supplemented with 50 ng/ml TPA (Sigma Catalogue No. P-8139) and 7% horse serum
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

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Subculturing: **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.
   An inoculum of 5 X 10(3) to 7 X 10(3) viable cells/sq. cm. is recommended.
6. Incubate cultures at 37°C.

Interval: Subculture when cells reach a concentration of 2 X 10(4) cells/sq. cm.
Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:4 is recommended
Medium Renewal: Two to three times weekly

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 29 hours

Related Products:
recommended serum:ATCC 30-2040
derived from same cell line:ATCC CRL-2771
Cell culture tested DMSO:ATCC 4-X
Erythrosin B vital stain solution:ATCC 30-2404
Trypan Blue vital stain solution:ATCC 30-2402

References:
89472: Govindarajan B, et al. Malignant transformation of melanocytes to melanoma by constitutive activation of mitogen-activated protein kinase kinase (MAPKK) signaling. J. Biol. Chem. 278: 9790-9795, 2003. PubMed: 12514183

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Cell Biology

ATCC® Number: CRL-1772™ Order this Item
Price: $256.00

Designations: C2C12

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: myoblast

PHOTO

Source:
Strain: C3H
Tissue: muscle
Cell Type: myoblast;

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 (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Comments: This is a subclone (produced by H. Blau, et al) of the mouse myoblast cell line established by D. Yaffe and O. Saxel. [22903]
The C2C12 cell line differentiates rapidly, forming contractile myotubes and producing characteristic muscle proteins. [22953]
Treatment with bone morphogenic protein 2 (BMP-2) cause a shift in the differentiation pathway from myoblastic to osteoblastic. [23427]
Tested and found negative for ectromelia virus (mousepox).

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

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Subculturing:

Protocol: IMPORTANT - DO NOT ALLOW CULTURES TO BECOME CONFLUENT.
Cultures must not be allowed to become confluent as this will deplete the myoblastic population in the culture.
Myotube formation is enhanced when the medium is supplemented with 10% horse serum instead of fetal bovine serum.

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 which 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.
   Inoculate at a cell concentration between 1.5 X 10 exp5 and 1.0 X 10 exp6 viable cells/75 cm2.
6. Incubate cultures at 37°C.

Medium Renewal: Every two to three days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
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:

22903: Yaffe D, Saxel O. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature 270: 725-727, 1977. PubMed: 563524
22953: Blau HM, et al. Plasticity of the differentiated state. Science 230: 758-766, 1985. PubMed: 2414846
23427: Katagiri T, et al. Bone morphogenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [published erratum appears in J Cell Biol 1995 Feb;128(4):following 713]. J. Cell Biol. 127: 1755-1766, 1994. PubMed: 7798324
28236: Chow YH, et al. Improvement of hepatitis B virus DNA vaccines by plasmids coexpressing hepatitis B surface antigen and interleukin-2. J. Virol. 71: 169-178, 1997. PubMed: 8985336
32828: Kessler PD, et al. Gene delivery to skeletal muscle results in sustanined expression and systemic delivery of a therapeutic protein. Proc. Natl. Acad. Sci. USA 93: 14082-14087, 1996. PubMed: 8943064
33069: Hsu DK, et al. Identification of a murine TEF-1-related gene expressed after mitogenic stimulation of quiescent fibroblasts and during myogenic differentiation. J. Biol. Chem. 271: 13786-13795, 1996. PubMed: 8662936

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

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

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: [signature]

BioSafety Officer(s): Stanley dept 21/09

Chair,Biohazards Subcommittee: [signature]

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 pannx1. 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: [signature]

BioSafety Officer(s): [signature] Sept 21/09

Chair,Biohazards Subcommittee: [signature]

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

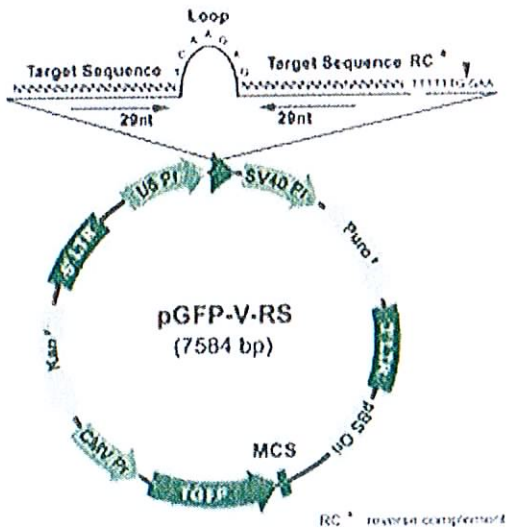
DNA Quantitation: The concentration of the purified plasmid was determined at OD_{260} by a UV spectrometer.

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



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Subject: Re: Biohazard Modification Form: Laird lab
From: Dale Laird <Dale.Laird@schulich.uwo.ca>
Date: Mon, 21 Sep 2009 08:24:54 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer, These vectors are for transfections only. We do not anticipate making viral particles with them. Dale

Dale W. Laird, Ph.D.
Professor
Canada Research Chair in Gap Junctions and Disease
Department of Anatomy and Cell Biology
University of Western Ontario
Dental Science Building, Rm 00077
London, Ontario, Canada, N6A-5C1
Tel: (519) 661-2111 x86827
Fax: (519) 850-2562
Dale.Laird@schulich.uwo.ca
www.uwo.ca/anatomy/laird/index.htm

>>> Jennifer Stanley <jstanle2@uwo.ca> 18/09/2009 5:19 pm >>>
Hi Dr. Laird:

Thank you for your recent Biohazard Modification Form submission. Please clarify, as soon as possible, whether or not the retrovirus particles are being produced.

Thanks,
Jennifer

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Cell Biology

ATCC® Number: CRL-6475™ Order this Item

Price: $264.00

Designations: B16-F10

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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 (CO_2), 5%

Subculturing:

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.

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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 (CO_2), 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 1 APRIL, 2009

Laird, Dale W. CIHR Operating Grant

Cx43 mutations linked to human disease

Intercellular gap junction channels allowing the direct passage of small metabolites and secondary messengers between contacting cells are built from a family 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, 62 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 to 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 co-expressed 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 co-expressed connexins remain unknown. Thus, we hypothesize that different ODDD-linked Cx43 mutants exhibit distinct cellular phenotypes and effects on co-expressed 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 intra-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) their ability to exert dominant and trans-dominant 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 co-express Cx43 or other connexins typically co-expressed with Cx43. *In vivo* FRET, co-immunoprecipitation and pulse-chase studies will be used to determine if the mutants establish direct interactions with co-expressed 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 harboring Cx43 gene mutations.

Aim 2: Characterize mouse models of ODDD harboring missense and truncation mutations in distinct regions of the Gja1 gene and analyze the consequence of the mutants on cell differentiation. Interestingly, our mouse models harboring different ODDD-linked mutants exhibit both similar and distinct phenotypes that are in the diversity of symptoms observed in ODDD patients. Thus, we will first finish the generation and characterization of "knock-in" genetically-altered mice in which the endogenous Cx43 coding sequence is replaced with the human wild-type Cx43 coding sequence or the $Cx43^{fs260}$ mutant coding sequence. Together with the $Cx43^{G60S}$, $Cx43^{I130T}$, $Cx43^{G138R}$ and the truncated mice, we will compare the phenotypes of these mutant mouse models of human ODDD by performing *in situ* analysis using combinations of micro-CT and micro-MRI analysis, in addition to immunohistochemistry of 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, and to muscle cells and myofibroblasts from the bladder, as approximately [illegible]% 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 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 ○ NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ○ 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	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500 ML	INVITROGEN	☒ 1 ○ 2 ○ 3
JM109	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	500 ML	PROMEGA	☒ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 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 ○ 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 ○ No	SKIN BIOPSIES	Not applicable
Rodent	☒ Yes ○ No	TRANSGENIC & MUTANT MICE	2006-101
Non-human primate	○ Yes ☒ No		
Other (specify)	○ 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 B.ICR. MIR, N2A	CLONETIC, ATCC CELL LINES, NASCALL
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.

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. S. GARIPEPU McGILL UNIVERSITY	CONNEXINS PANNEXINS	CELLS COMMUNICATE BETTER

* Please attach a Material Safety Data Sheet or equivalent. SEE B.O-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:

_____
_____
_____

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

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 pathogenic 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:

-------- 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