Modification Form for Permit RIO-LHRI-0089

Permit Holder: John Lewis

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

Alex Mellulis
Rae Nesbitt
Catalina Vasquez
Shruti Nambiar
Fong Cho
Laura Fung
Amber Ablack
Amy Robertson
Hon Leong

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	Lentivirus	
Approved Primary and Established Cells	[Established, Human] - HT-1080, HEK293 phoenix cells.	
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	[vectors] - pLvx-puro-EGFP, MSCV-LTRmiR30-P16.	FUtdTW, FUmGW, pLKO.1-TRC, pmAmetrine-DEND-dTomato
Approved Use of Animals	Chicken Embryo	
Approved Biological 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 STORED, USED AND DISPOSED OF.*

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: _____

Current Classification: 2+ **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Jan 18, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____ Aug 23/10

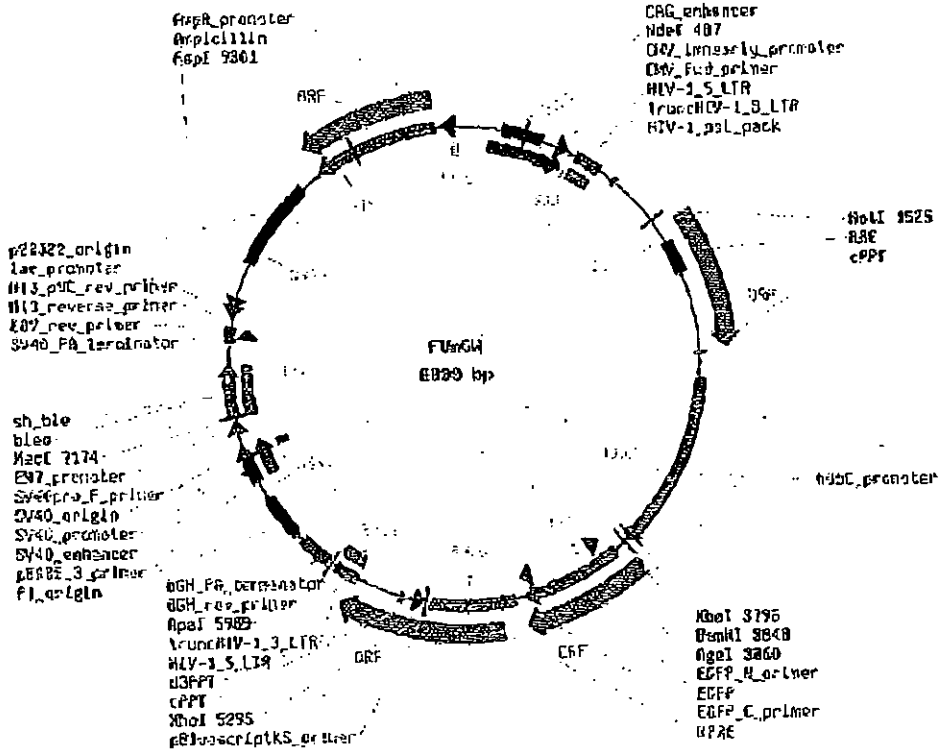
Chair, Biohazards Subcommittee: _____ **Date:** _____

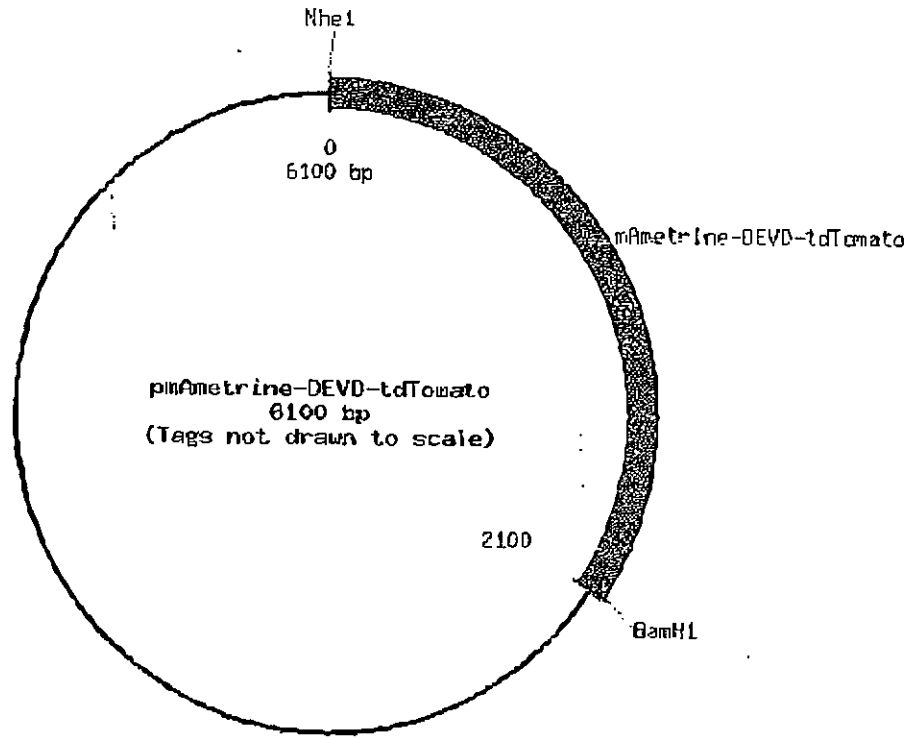
Description of work that will use the requested plasmids (being ordered via Addgene):

1. FUtdTW plasmid: this plasmid will be used to make HT-1080 cancer cells produce fluorescent red protein and then these cancer cells will be injected intravenously into the chicken embryo and imaged for 2-4 days.
2. FUmGW plasmid: this plasmid will be used to make HT-1080 cancer cells produce fluorescent green proteins and then these cancer cells will be injected intravenously into the chicken embryo and imaged for 2-4 days.
3. pLKO.01-TRC: this plasmid will be used to make virus that contains no genetic construct and will be used as a negative control because it has no additional genetic insert. Virus will be used to infect HT1080 cells and then injected intravenously into the chicken embryo and imaged for 2-4 days.
4. pmAmetrine-DEVD-tdTomato: this plasmid will be used to transfect HT1080 cells (no virus being made or used for this plasmid) and then these cells will be injected intravenously into the chicken embryo and imaged for 2-4 days.

Hi Jennifer,
This is the work summary for the new plasmids we are ordering.

Thanks,
[illegible]
57193





THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
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 Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must 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, Public Health Agency of Canada (PHAC) 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 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR: John Lewis
SIGNATURE:
DEPARTMENT: Oncology
ADDRESS: LRCP Rm A4-823
PHONE NUMBER: x57194 3
EMERGENCY PHONE NUMBER(S): 519-200-1021
EMAIL: john.lewis@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LRCP Room(s) A4-823 A4-822(TC)

*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 the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES:
GRANT TITLE(S): Migration-mediated intravasation and tumour cell metastasis in the dissemination of cancer. Non-invasive imaging of pathological angiogenesis using targeted multivalent nanoparticles.

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Amber Ablack
Laura Fung
Fong Cho
Bala Iyengar
Non Leong
Smruti Nambiar
Catalina Vasquez
Rae Nesbitt
Amy Robertson
Alex Meiluts

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ⊘ YES O NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)?
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	PHAC or CFIA Containment Level
Lentivirus	⊘ Yes O No	⊘ Yes O No	O Yes ⊘ No	0.02L	~~various~~ clontech	O 1 ⊘ 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
	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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes ⊘ No		Not applicable
Rodent	O Yes ⊘ No		
Non-human primate	O Yes ⊘ No		
Other (specify)	⊘ Yes ~~⊘ No~~	chicken embryo	

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

2.3 Please indicate the type of established cells that will be grown in culture in: HEK 293 Phoenix cells

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☑ Yes O No	HT-1080 *	all cell lines from ATCC.
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

I'll email attached

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☑ 1 O 2 O 3

*please see attached sheet

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O 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)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid	N/A	O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 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 O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☑ YES, complete table below * O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

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

*please see attached.

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Lentivirus	pLVX-puro-EGFP pMSCV-LTRmiR30-PIG IDT	clontech,	EGFP, shRNA's (5)	cells turn green, less mobile cells.

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV ☑ YES, please specify HIV-1 O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ☒ NO
- SV 40 Large T antigen O YES ☒ NO
- E1A oncogene ✓ YES ~~O NO~~ ☒ NO
- Known oncogenes O YES, please specify _____ ☒ NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☒ NO

HKK293 cells

4.5 Will virus be replication defective? ☑ YES O NO

4.6 Will virus be infectious to humans or animals? ☑ YES O NO

4.7 Will this be expected to increase the containment level required? ☑ YES O NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ☑ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used chicken embryo.

6.3 AUS protocol # 2007-087-10

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ ☑ NO

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

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 Ø NO
- Pound source cats O YES Ø NO
- Cattle, sheep or goats O YES Ø NO
- Non-human primates O YES, please specify species _____ Ø NO
- Wild caught animals O YES, please specify species & colony # _____ Ø NO
- Birds (embryonic) - Ø YES O NO
- Others (wild or domestic) O YES, please specify _____ Ø NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø 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_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø 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 life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" 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
If NO, please forward the permit to he Biosafety Officer when available.

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 12.0 ☒ 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 plant 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.

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

SIGNATURE _____

13.0 Containment Levels

level 2+ email attached 2l.

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 ☒ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

☒ YES, permit # if on-campus_____

NO, please certify

O NOT REQUIRED for Level 1 containment

Room A4-822 LRCP Certified Level 2 by me on Nov. 25, 2009. Jaime Ryder

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: Aug 6/09.

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: G.M. Kidder
Date: 18 Jan. 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: Jaime Ryder
Date: December 21, 2009

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: J Stanley.
Date: Jan 18, 2010

Approval Number: BIO-LHR-0089 Expiry Date (3 years from Approval): January 17, 2013

Special Conditions of Approval:

- Viral Vector Policy attached (to be followed).

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

Description of the project involving biohazardous agent

The goal of this project is to screen for targets of migration in HT-1080 cancer cells. Isolation of these genetic targets can be achieved by introduction of short-hairpin RNA (shRNA) against all the genes in the human genome, into cancer cells. The best way to incorporate these shRNAs into the nucleus of the cell is to put them into a lentiviral vector. The nature of lentiviruses allows for the infection of both dividing and non-dividing cells and permits integration of the shRNA into the cellular DNA . Thus, lentiviral infection is best suited for our applications.

The result of a lentiviral infection will allow for an assortment of HT-1080 cells that stably express the shRNA against a given gene. Placement of these cancer cells within a chicken embryo will allow for the detection of non-migratory tumor types. Furthermore, the isolation of these tumor types will reveal the genes affected by the shRNA and, ultimately, contribute to migration.

The lentiviral vector we will use for delivery of the shRNA is replication defective and, therefore, does not replicate within its host. The production of lentivirus will be achieved by transfection of a packaging cell line, Hek 293 with the lentiviral vector containing the shRNA. Once transfected, the packaging cells will create lentivirus particles which can be used to infect HT-1080 cells.

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The Global Bioresource Center™

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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: CCL-121™ Order this Item
Price: $264.00

Designations: HT-1080

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source:
Tissue: connective tissue
Disease: fibrosarcoma

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:** July, 1972

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

Virus Susceptibility:
Human poliovirus 1
RD-114 Feline
Feline leukemia virus
Vesicular stomatitis virus

Tumorigenic: Yes

Reverse Transcript: negative

Oncogene: ras +

DNA Profile (STR):
Amelogenin: X,Y
CSF1PO: 12
D13S317: 12,14
D16S539: 9,12
D5S818: 11,13
D7S820: 9,10
THO1: 6
TPOX: 8
vWA: 14,19

Cytogenetic Analysis: modal number = 46; range = 44 to 48.
Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.

Isoenzymes: G6PD, B

Age: 35 years

Gender: male

Ethnicity: Caucasian

Related Links
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Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Comments: The cells contain an activated N-ras oncogene.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. 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

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 1:4 to 1:8 is recommended
Medium Renewal: Every 2 to 3 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 30-2003
recommended serum:ATCC 30-2020

References:

22147: Chen TR, et al. Intercellular karyotypic similarity in near-diploid cell lines of human tumor origins. Cancer Genet. Cytogenet. 10: 351-362, 1983. PubMed: 6652615
23071: Geiser AG, et al. Suppression of tumorigenicity in human cell hybrids derived from cell lines expressing different activated ras oncogenes. Cancer Res. 49: 1572-1577, 1989. PubMed: 2647289
23393: Rasheed S, et al. Characterization of a newly derived human sarcoma cell line (HT-1080). Cancer 33: 1027-1033, 1974. PubMed: 4132053
25969: Adams RA, et al. Direct implantation and serial transplantation of human acute lymphoblastic leukemia in hamsters, SB-2. Cancer Res. 28: 1121-1125, 1968. PubMed: 4872716
26035: . . Proc. Am. Assoc. Cancer Res. 8: 1, 1967.
32289: Hu M, et al. Purification and characterization of human lung fibroblast motility-stimulating factor for human soft tissue sarcoma cells: identification as an NH2-terminal fragment of human fibronectin. Cancer Res. 57: 3577-3584, 1997. PubMed: 9270031
32370: Iida A, et al. Inducible gene expression by retrovirus-mediated transfer of a modified tetracycline-regulated system. J. Virol. 70: 6054-6059, 1996. PubMed: 8709228
32531: Brenneman M, et al. Stimulation of intrachromosomal homologous recombination in human cells by electroporation with site-specific endonucleases. Proc. Natl. Acad. Sci. USA 93: 3608-3612, 1996. PubMed: 8622983
33061: Seiffert D. Hydrolysis of platelet vitronectin by calpain. J. Biol. Chem. 271: 11170-11176, 1996. PubMed: 8626663
33152: Hocking AM, et al. Eukaryotic expression of recombinant biglycan. J. Biol. Chem. 271: 19571-19577, 1996. PubMed: 8702651

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Back to my Search

Hi Jennifer,

In vivo work with lentiviruses should be conducted in a containment level 2 physical laboratory with the use of containment level 3 operational practices. Work in chicken embryos is still considered in vivo in this case.

Kind regards and happy holidays

Genevieve

Genevieve Lacroix, M.Sc.
Head, Importation and Biosafety Programs/ Chef, Importation et services de biosécurité
Pathogen Regulation Directorate (formerly Office of Laboratory Security) / Direction de la réglementation des agents pathogènes (anciennement le Bureau de sécurité des laboratoires)
Public Health Agency of Canada / Agence de la santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
Tel: (613) 946-6932
Fax: (613)941-0596
genevieve.lacroix@phac-aspc.gc.ca
http://www.phac-aspc.gc.ca/ols-bsl/index.html

Jennifer Stanley <jstanle2@uwo.ca>
2009-12-22 05:36 PM

To
genevieve.lacroix@phac-aspc.gc.ca
cc

Subject
Containment Level request - lentiviral project

The goal of this project is to screen for targets of migration in HT-1080 cancer cells. Isolation of these genetic targets can be achieved by introduction of short-hairpin RNA (shRNA) against all the genes in the human genome, into cancer cells. The best way to incorporate these shRNAs into the nucleus of the cell is to put them into a lentiviral vector. The nature of lentiviruses allows for the infection of both dividing and non-dividing cells and permits integration of the shRNA into the cellular DNA. Thus, lentiviral infection is best suited for our applications.

The result of a lentiviral infection will allow for an assortment of HT-1080 cells that stably express the shRNA against a given gene. Placement of these cancer cells within a chicken embryo will allow for the detection of non-migratory tumor types. Furthermore, the isolation of these tumor types will reveal the genes affected by the shRNA and, ultimately, contribute to migration.

The lentiviral vector we will use for delivery of the shRNA is replication defective and, therefore, does not replicate within its host. The production of lentivirus will be achieved by transfection of a packaging cell line, Hek 293 with the lentiviral vector containing the shRNA. Once transfected, the packaging cells will create lentivirus particles which can be used to infect HT-1080 cells.

Subject: Re: Biohazardous Agents Registry Form: Lewis
From: Gail Ryder <Gail.Ryder@LawsonResearch.Com>
Date: Wed, 23 Dec 2009 15:03:11 -0500
To: Jennifer Stanley <jstanle2@uwo.ca>

Yes it is. That room is a dedicated, stand-alone lentivirus room. All users are trained in Level 2 plus level 3 precautions as well.

Gail

Gail Ryder, CRSP
Research Safety Officer

Lawson Health Research Institute
South Street Hospital
375 South Street, Room A210, NR
London, Ontario, Canada N6A 4G5
Tel: (519) 685-8500 x75109
Fax: (519) 432-7367
Pager: x18059
E-mail: Gail.Ryder@LawsonResearch.com
Website: www.lawsonresearch.com

Jennifer Stanley <jstanle2@uwo.ca> 2009/12/23 02:32 PM >>>

Hi Gail:

I received the Lewis Biohazardous Agents Registry Form. The project involves work using lentiviral vectors. This type of work is normally Level 2 plus Level 3 precautions. Is the room A4-822 set up for that?

Thanks,
Jennifer

-------- Original Message --------
Subject: Re: Bioahzardous Agents Registry Form: Lewis
Date: Fri, 15 Jan 2010 18:04:11 -0500
From: Amy Atkinson <amyeatkinson@gmail.com>
To: Jennifer Stanley <jstanle2@uwo.ca>

Jennifer,

We will be using pheonix cells, HEK 293, and 'Boss Cells' for any of the lenti work we will be doing. I was going to email you soon to see what our status is with the certification. Would you know the answer or should I email Gail Ryder instead?

Thanks,
Amy

Policy on Research Utilizing Virus Vector Transduced Cells or Virus Infection of Animals
Version 5
Approved by Biosafety Committee: June, 2009

Research with cells transduced with replication competent or defective viral vectors capable of infecting human or animal cells must be carried out in an approved Containment Level 2 (CL2) physical laboratory. This includes, but is not limited to vectors derived from Adenovirus, Adeno-associated virus, lab adapted strains of Vesicular Stomatitis Virus, alpha viruses, measles virus, murine, avian or feline gamma retroviruses (formerly known as type C retroviruses) and herpes simplex virus type I or II. Even though the gamma retroviral vector may be replication defective, endogenous retroviruses residing within the transduced cells *in vitro* or *in vivo* could package the nascent viral RNA as pseudotyped infectious particles. Both amphotropic and xenotropic retroviruses from different species are capable of infecting human cells. Research utilizing replication defective lentiviral vectors must be conducted in a Containment Level 2 (CL2) physical laboratory with the use of Containment Level 3 (CL3) operational practices (commonly termed CL2+). This includes vectors derived from, but not limited to, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). Researchers are strongly encouraged to use self-inactivating lentiviral vectors. These guidelines also apply to *in vivo* work.

Research involving a live replication competent or defective viral vector containing a known oncogene, regardless of the type of the viral vector, requires CL3 if the vector is infectious for human cells. Viral vectors expressing genes that are known to be anti-apoptotic or promote cell survival and/or proliferation may also require higher levels of containment but will have to assessed on a case by case basis by the UWO Biohazards Subcommittee.

It is recognized that experiments involving direct injection of virus or a virus-transduced cell line into an animal place significant burden on the researchers in order to meet the recommended guidelines. For example, conducting a stereotaxic injection of a viral vector into a targeted area of the brain is generally not possible using conventional laminar flow hoods. Whole animal imaging (MRI, CT, PET or ultrasound, bioluminescence) and flow cytometry of live vector-transduced cells are additional examples where biosafety issues make experimental protocols more difficult. In an effort to help reduce this burden, the following procedures are proposed to provide proof that no virus is being released from transduced cells as a way to reduce the need for CL2 or CL2+ containment.

Gamma retrovirus or lentivirus vectors:

For experiments that require that cells stably transduced with a gamma retroviral or lentiviral vector be injected into an animal the level of containment can be dropped providing the following conditions can be satisfied:

1. The use of self-inactivating gamma retroviral or lentiviral vectors is strongly advised when available. Commercially available lentiviral vectors are self-inactivating. Most gamma retroviral vectors are not.

2. Once stable viral transductants have been selected/established under the required containment conditions, the engineered cells containing a reporter gene (GFP or luciferase for example), a gene that mediates targeted recombination (Cre or Flip recombinase) or a gene that modifies metabolism but does not affect the cell cycle or proliferation can be tested for the absence of virus production. This can be demonstrated by taking the clarified cell supernatant from the transduced cell line after 5 to 10 cell passages and adding it to cultures of the original uninfected cells or a similar cell line that is highly permissible to viral infection. Reporter gene assays can then be conducted after 48 to 72 hours of culture. However, these types of assays may not be particularly sensitive and should be discussed with the Biohazard Subcommittee in advance. The preferred approach, and that which must be done for all non-reporter gene constructs, is to use quantitative PCR as the confirmatory assay with appropriate standards to confirm assay sensitivity. The assay must be sensitive enough to detect at least one infected cell per 10^6 uninfected cells. Alternatively, clarified supernatants from cell passage 5 to 10 can be concentrated by ultracentrifugation and the pellet area extracted in the presence of carrier RNA. Real time qRT-PCR can be conducted with standards to determine if virus is being released from the stably transduced cells. In either case one primer should be derived from the vector sequence and the other from the transgene of interest. If the virus is undetectable in either of these assays, a CL2 or CL2+ cell line could be handled at its original, nontransduced containment level. Animals injected with these reclassified cells could also be handled at their original, nontransduced containment levels. If gamma retro virus or lentivirus vectors must be injected directly into animals then injections can be conducted in a level 2 room outside of a laminar flow hood provided appropriate personal protective equipment is worn and appropriate decontamination procedures are in place. Once this proof of principle experiment is conducted and submitted to the Biohazard Subcommittee for review, then all subsequent experiments using the same gamma retroviral or lentiviral vector transduced cells can be done under reduced containment. Positive detection of the virus in culture supernatant or as integrated viral DNA from test cells would require maintenance of the virally transduced containment level.

Note that this "dropdown" option does not apply to immunocompromised mice repopulated with primary human or nonhuman primate (NHP), unmodified primary or viral vector modified primary cells. For those mice, the containment must not be lower than CL2 (the standard for handling any primary human material) or CL2+ (the standard for handling NHP material). If the primary cells are known to be infected with a risk group 3 human pathogen, then they must be handled at the containment level appropriate for that pathogen. If the transduced gene is known to promote cell survival or alter cell cycling in favour of proliferation (as in the case of an oncogene), then CL2+ or a higher containment level, determined by a risk assessment made in collaboration with the Biohazard Subcommittee, must be maintained for live viral vector work, especially if the vectors are capable of infecting human cells.

Adenovirus vectors:

For animal experiments that require the use of replication competent adenovirus vectors (first generation vectors), level 2 containment must be observed regardless of the transgene to be used. For experiments using 2^{nd} or 3^{rd} generation replication defective Adenovirus vectors that do not contain an oncogene or genes that promote cell survival and or cell proliferation, direct injection

of virus infected cells or direct injection of virus can be done outside a laminar flow hood in an approved level 2 room with personal protective equipment worn once the following proof of principle condition has been satisfied:

Following injection of the animal, bodily fluids such as blood, bronchial lavage, and urine as well as stool should be collected at several time points over the first 14 days post-infection. Quantitative PCR with the use of positive spiking controls and assay sensitivity controls can then be used to demonstrate that the recombinant Adenovirus is not being released from the infected animal. Once this proof of principle experiment is conducted then all following experiments using the same Adenovector can be done under reduced containment conditions and the animals can be returned to CL1 animal housing at the point when the Q-PCR gave reproducible negative results.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

Adeno-associated virus vectors:

For experiments using recombinant Adeno-associated virus vectors it is strongly recommended that the vector be generated using a construct that can generate the vector by transfection such that helper virus is not required. For direct animal injection experiments the same proof of principle experiment as described for the Adenovirus vectors must be conducted before lowering of the containment level for animal housing can be considered.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

Other viral vectors:

Experiments requiring the use of less commonly used viral vectors will need to be considered by the Biohazard Subcommittee on a case by case basis in consultation with AUS-ACVS.