

Lewis
BIO-LHR1-0089

Modification Form for Permit B10-LHR1-0089

Permit Holder: John Lewis

Approved Personnel

(Please stroke out any personnel to be removed)

- Alex Meilutis
- Rae Nesbitt
- Catalina Vasquez
- Shnuli 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] - ~~XXXXXXXXXX~~
PWPXL

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.wvph.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): Aug 25, 2010

BioSafety Officer(s): (Peter Ferguson, PhD)

for Chair, Biohazards Subcommittee: Peter Ferguson

Date: May 26, 2011

Description of the work involving this plasmid

This vector will be used to treat cancer cells (HT1080) as a negative control. There are no genes in this vector that will be encoded by the vector. It is simply being used as a negative control for non-expression. Controls of this type are called "empty vector".

 **Plasmid 12257: pWPXL** 

Gene/insert name: None
Insert size (bp): Unknown
Fusion proteins or tags: EGFP
Terminal: Unknown
Vector backbone: pWPXL
([Search Vector Database](#))
Type of vector: Mammalian expression,Lentiviral,Cre/Lox
Backbone size (bp): 10510
Cloning site 5': MCS - see map
Site destroyed during cloning: No
Cloning site 3': MCS - see map
Site destroyed during cloning: No
5' Sequencing primer: See map ([List of Sequencing Primers](#))
Bacteria resistance: Ampicillin
High or low copy: High Copy
Grow in standard E. coli @ 37C: No
Please specify bacterial strain for growth and growth condition: Use Stb13 or HB101 to reduce chance of recombination. Grow at 37C
Sequence: [View sequence](#)
Author's Map: [View map](#)
Plasmid Provided In: Stb13
Principal Investigator: Didier Trono
Terms and Licenses: [MTA](#)

Comments: pWPT or pWPXL can be used for constitutive transgene expression. pWPXL contains the EF-1alpha promoter + intron that gives you high expression, as RNA loves to be spliced (it goes more efficiently out of the nucleus). pWPT contains only the EF-1alpha promoter

The loxP site in the 3'LTR is duplicated to the 5'LTR during reverse transcription in the target cells. This allows for removal (if necessary) of an integrated provirus by Cre.

Unique restriction sites at key positions will allow you to change promoter and transgene. PmeI is a popular site for cloning. You can also use PacI and SwaI.

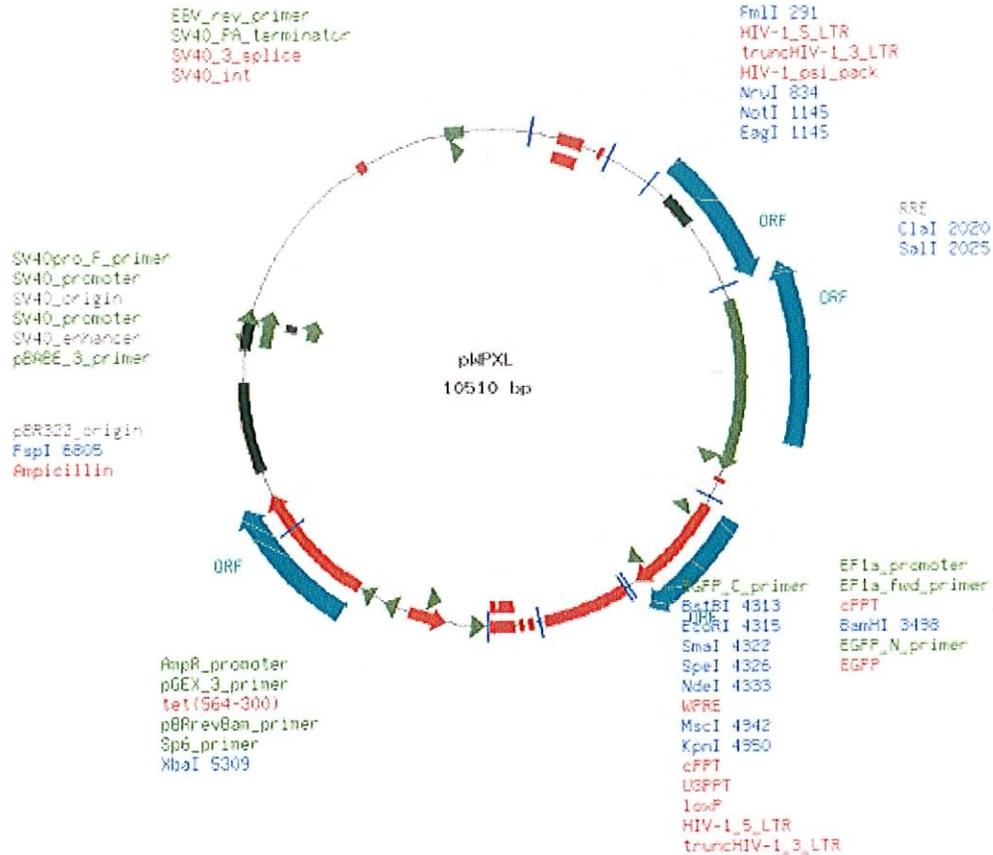
Please note that ClaI in this vector is blocked by Dam methylation. This plasmid needs to be grown in a Dam- bacteria strain if you wish to use ClaI for cloning.

Packaging plasmids for Trono lab lentiviral vectors are also available at Addgene (<http://www.addgene.org/rnaitools>).

Please note that the full sequence for this plasmid is approximated and not fully verified. Please visit the Trono lab (<http://tronolab.epfl.ch>) for cloning strategies, protocols, publications, and more. See LentiWeb (<http://www.lentiweb.com>) for discussions on cloning strategies and protocols.

Addgene has sequenced a portion of this plasmid for verification. Click [here](#) for the sequencing result.

Click on map to enlarge



Selected features

HIV-1_5_LTR	455 - 635	
truncHIV-1_3_LTR	455 - 635	
HIV-1_psi_pack	746 - 790	
RRE	1300 - 1533	
ORF frame 2	1178 - 2035	
ORF frame 2	3037 - 1997	
EF1a_promoter	2127 - 3297	
EF1a_fwd_primer	3245 - 3265	
cPPT	3349 - 3364	
EGFP_N_primer	3610 - 3589	

Unique restriction sites

PmlI	291
NruI	834
NotI	1145
EagI	1145
ClaI	2020
Sall	2025
BamHI	3498
BstBI	4313
EcoRI	4315
SmaI	4322

EGFP	3544 - 4260		SpeI	4326
ORF frame 1	3544 - 4281		NdeI	4333
EGFP_C_primer	4197 - 4218		MscI	4942
WPRE	4342 - 4929		KpnI	4950
cPPT	5000 - 5015		XbaI	5309
U3PPT	5000 - 5021		FspI	6805
loxP	5072 - 5105			
HIV-1_5_LTR	5125 - 5305			
truncHIV-1_3_LTR	5125 - 5305			
Sp6_primer	5348 - 5331			
pBRrevBam_primer	5657 - 5638			
tet(564-300)	5849 - 5586			
pGEX_3_primer	5990 - 6012			
AmpR_promoter	6171 - 6199			
ORF frame 1	6241 - 7101			
Ampicillin	6241 - 7101			
pBR322_origin	7256 - 7875			
pBABE_3_primer	8121 - 8101			
SV40_enhancer	8322 - 8107			
SV40_promoter	8119 - 8387			
SV40_origin	8286 - 8363			
SV40_promoter	8239 - 8441			
SV40pro_F_primer	8348 - 8367			
SV40_int	9568 - 9583			
SV40_3_splice	9589 - 9636			
SV40_PA_terminator	10212 - 10343			
EBV_rev_primer	10300 - 10319			

Please acknowledge the principal investigator if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 12257" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Modification Form for Permit BIO-LETRE-0089
Permit Holder: John Lewis

Approved Personnel

(Please stroke out any personnel to be removed)

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

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(Please list additional personnel here)

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Approved Microorganisms

Lentivirus

Approved Primary and Established Cells

[Established, Human] - HIT-1080, HEK293 phoenix cells.

Approved Use of Human Source Material

Approved Genetic Modifications (Plasmids/Vectors)...

[vectors] - pLvX-puro-EGFP, MSCV, LTRmiR30-P16.

FUtdTW, FUmGW, pKO.1-TRC, pm Ametrine-DEVD-ttTowato

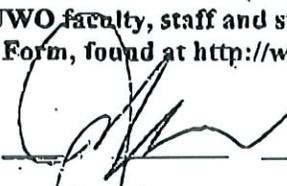
Approved Use of Animals

Chicken Embryo

Approved Biological Toxin(s)

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** 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: Level 2⁺

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

Date of Last Modification (if applicable):

BioSafety Officer(s):

Maire Ryan Aug 23/10

J Stanley Oct 7/10

Chair, Biohazards Subcommittee:

M Miller

Date: OCT 13 2010

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 ^{lentivirus} 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 ^{lentivirus} 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.

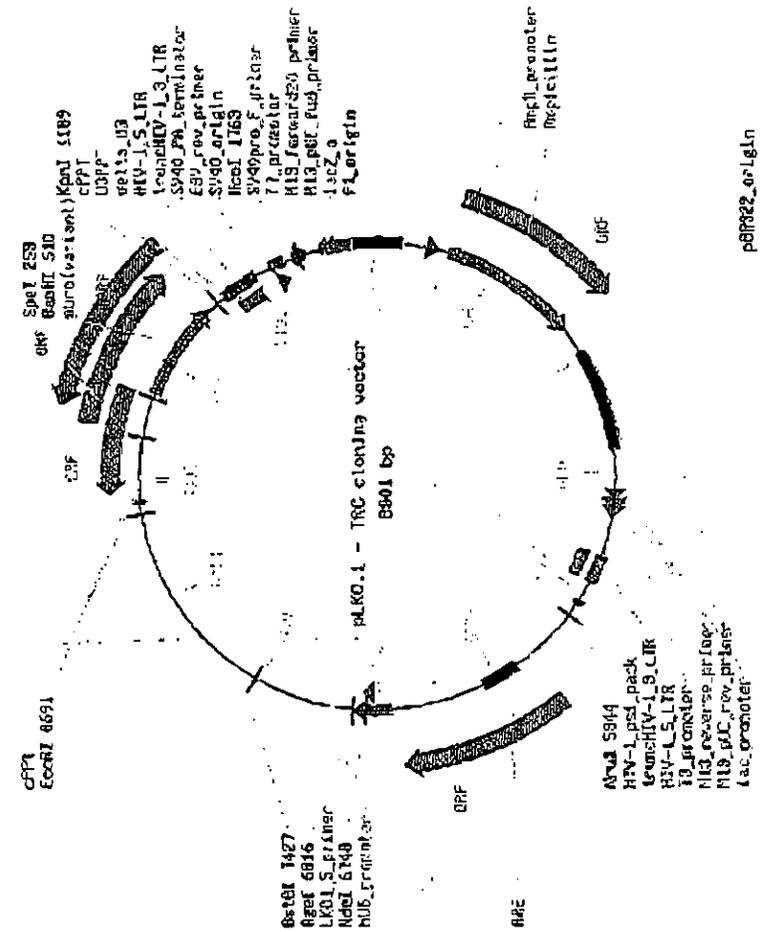
Hi Jennifer,

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

Thanks,

Jon

57193



RECEIVED 08-23-'10 12:07

FROM- 5194327367

TO- UWO-HR-Occ. Health

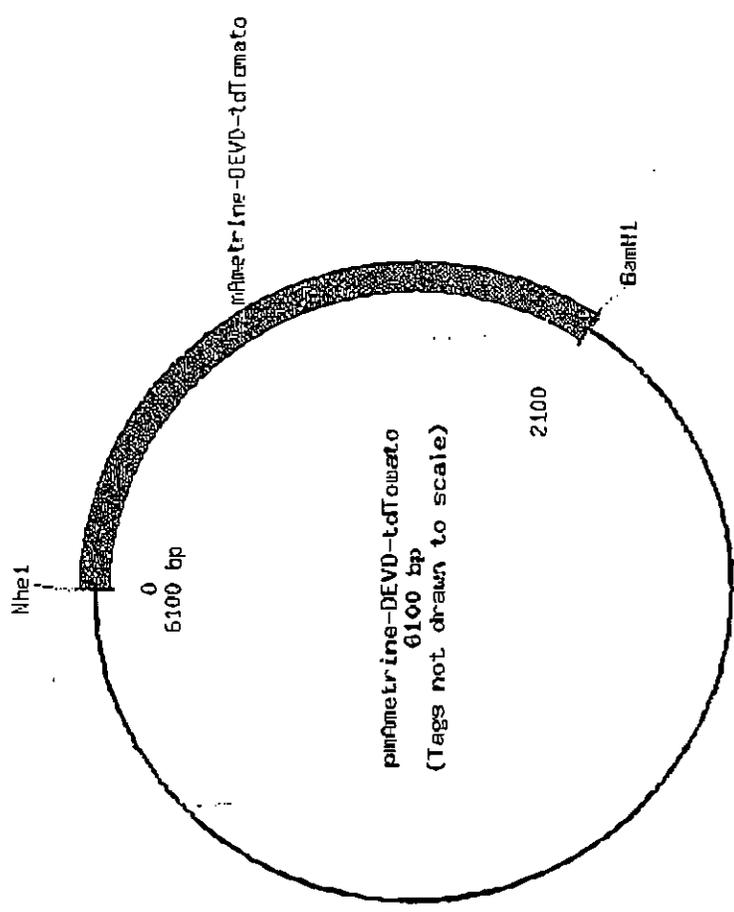
FROM- 5194327367

FROM-LAWSON HEALTH RESEARCH 1

519 432 7367

1-773 P:007/007 F-341

23-AUG-2010 12:11



**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
SIGNATURE
DEPARTMENT
ADDRESS
PHONE NUMBER
EMERGENCY PHONE NUMBER(S)
EMAIL

John Lewis
Oncology
LRCR Rm A4-823
571943
519-206-1021
john.lewis@hsc.uwo.ca

Location of experimental work to be carried out: Building(s) LRCR P 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:

<u>Amber Ablack</u>	<u>Shruti Nambiar</u>
<u>Laura Fung</u>	<u>Catalina Vasquez</u>
<u>Long Cho</u>	<u>Rae Nesbitt</u>
<u>Bala Tyengar</u>	<u>Amy Robertson</u>
<u>Non Heong</u>	<u>Alex Meilutis</u>

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES 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? 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.02L	various clontech	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No	chicken embryo	

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

HEK 293
Phoenix cells

Jal
email
attached

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

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

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

*please see attached sheet

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)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	NA	<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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

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

*please see attached.

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Lentivirus	PLVX-puro-EGFP MSCV-LTR-mir30-PI6 1D1	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 NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

HK293 cells

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 involving a biological agent? 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? YES 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? 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 chicken embryo

6.3 AUS protocol # 2007-087-10

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? 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? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES 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? YES NO
If NO, please forward the permit to the 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? 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? YES NO

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

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ 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 _____

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.

1 2 3

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

YES, permit # if on-campus _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

level 2+
email attached
pl.
Room A4-822 LRCP
certified level 2 by
me on Nov. 25, 2009.
Maile Ryden

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: [Signature]
Date: 18 Jan. 2010

Safety Officer for Institution where experiments will take place:

SIGNATURE: [Signature]
Date: December 21, 2009

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

SIGNATURE: [Signature]
Date: Jan 18, 2010

Approval Number: B10-LHR1-0089 Expiry Date (3 years from Approval): January 17, 2013

Special Conditions of Approval:

• Viral Vector Policy attached. (to be followed).

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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Product Description

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Cell Biology

ATCC® Number:	CCL-121™	<input type="button" value="Order this Item"/>	Price:	\$264.00
Designations:	HT-1080			
Biosafety Level:	1			
Shipped:	frozen			
Medium & Serum:	See Propagation			
Growth Properties:	adherent			
Organism:	<i>Homo sapiens</i> (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 TH01: 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			

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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: <ol style="list-style-type: none"> 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. <p style="text-align: center;">Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended Medium Renewal: Every 2 to 3 days</p>
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. <i>Cancer Genet. Cytogenet.</i> 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. <i>Cancer Res.</i> 49: 1572-1577, 1989. PubMed: 2647289 23393: Rasheed S, et al. Characterization of a newly derived human sarcoma cell line (HT-1080). <i>Cancer</i> 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. <i>Cancer Res.</i> 28: 1121-1125, 1968. PubMed: 4872716 26035: . . . <i>Proc. Am. Assoc. Cancer Res.</i> 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 NH ₂ -terminal fragment of human fibronectin. <i>Cancer Res.</i> 57: 3577-3584, 1997. PubMed: 9270031 32370: Iida A, et al. Inducible gene expression by retrovirus-mediated transfer of a modified tetracycline-regulated system. <i>J. Virol.</i> 70: 6054-6059, 1996. PubMed: 8709228 32531: Brennenman M, et al. Stimulation of intrachromosomal homologous recombination in human cells by electroporation with site-specific endonucleases. <i>Proc. Natl. Acad. Sci. USA</i> 93: 3608-3612, 1996. PubMed: 8622983 33061: Seiffert D. Hydrolysis of platelet vitronectin by calpain. <i>J. Biol. Chem.</i> 271: 11170-11176, 1996. PubMed: 8626663 33152: Hocking AM, et al. Eukaryotic expression of recombinant biglycan. <i>J. Biol. Chem.</i> 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.
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Pathogen Regulation Directorate (Formerly Office of Laboratory Security) /
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Jennifer Stanley <jjstanle2@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

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||| 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

This information is directed in confidence solely to the person named above and may contain confidential and/or privileged material. This information may not otherwise be distributed, copied or disclosed. If you have received this e-mail in error, please notify the sender immediately via a return e-mail and destroy original message. Thank you for your cooperation.

----- Original Message -----

Subject:Re: Bioahazardous 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 be 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 2nd or 3rd 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 CLI 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.