Modification Form for Permit BIO-UWO-0151

Permit Holder: Marc Tini

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

Debbie Dong

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Replication-defective Moloney murine leukemia virus, E. coli, Replicative-defective Baculovirus	
Approved Cells	[Rodent] - Mouse embryo fibroblast (AUS 2006-086-08), (established): Human (established), HeLa, NIH3T3, MCF-7, cos7	
Approved Use of Human Source Material	Brain tumour tissue bank, ontario tumour bank	
Approved GMO	Moloney murine leukemia virus, baculovirus (pSUPER, pLNCX, pFastBAC), [Plasmid] PCMX, PET,pLNCX pGEX. [Vector] - pLNCX	pCF CREB, pBABEpuro SV40 large T antigen
Approved use of Animals		
Approved Toxin(s)		

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.
** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: [signature]

Classification: 2

Date of Last Biohazardous Agents Registry Form: Jun 26, 2009

Date of Last Modification (if applicable): ________

BioSafety Officer(s): ________

Chair, Biohazards Subcommittee: ________

Work being done with new agents

pBABEpuro SV40 large T antigen

Moloney murine leukemia virus based vector for expression the SV40 large T antigen. Ecotropic virus will be generated and used to immortalize mouse embryonic fibroblasts. Virus is replication deficient.

pCF CREB

Mammalian expression vector for CREB transcription factor. This vector will transfected into mammalian cells and the activity of the CREB transcription factor will be monitored by reporter gene assays.

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Browse > Thomas Roberts > Article > pBABE-puro SV40 LT

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Plasmid 13970: pBABE-puro SV40 LT

Gene/insert name:	SV40 LT
Alternative names:	Simian virus-40 large-T antigen
Insert size (bp):	2200
GenBank/Entrez ID of insert:	AF316141
Gene/insert aliases:	SV40gp6
Species of gene(s):	H. sapiens (human) Other
Vector backbone:	pBABE-puro (Search Vector Database)
Type of vector:	Mammalian expression,Retroviral
Backbone size (bp):	5169
Cloning site 5':	BamHI?
Site destroyed during cloning:	No
Cloning site 3':	BamHI?
Site destroyed during cloning:	No
5' Sequencing primer:	pBABE 5' (List of Sequencing Primers)
3' Sequencing primer:	pBABE 3'
Bacteria resistance:	Ampicillin
High or low copy:	Unknown
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Puromycin
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Thomas Roberts
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge

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Price: $65.00

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- From this article
- SV40gp6 plasmids
- Thomas Roberts Lab Plasmids

Other Links
- AF316141
- NCBI: SV40gp6

This is commonly requested with
- pBABE-hygro-hTERT
- pMIG-hOCT4
- pMIG-hSOX2
- pMIG-hKLF4
- pCMV-VSV-G

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Plasmid 13970

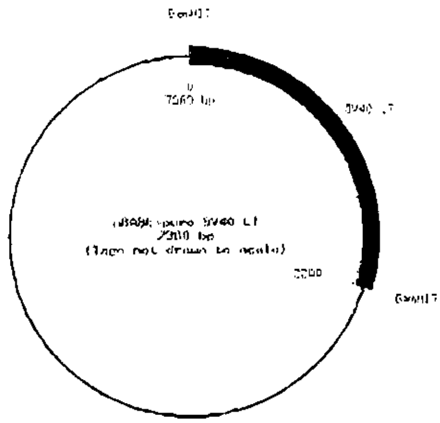
pBABE-puro
Plasmid 1764

pMSCVgfp::AID
Plasmid 15925

Nina Papavasiliou Lab Plasmids

CMV-Sp1
Plasmid 12097

More



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

Also please include the text "Addgene plasmid 13970" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

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Browse > Marc Montminy > Du et al > pCF CREB

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Plasmid 22968: pCF CREB

Gene/insert name:	CREB
Insert size (bp):	Unknown
Gene/insert aliases:	Creb1, Creb
Species of gene(s):	R. norvegicus (rat)
Fusion proteins or tags:	FLAG
Terminal:	N terminal on insert
Vector backbone:	pCF (Search Vector Database)
Type of vector:	Mammalian expression
Backbone size (bp):	5000
Cloning site 5':	EcoRI
Site destroyed during cloning:	No
Cloning site 3':	XbaI
Site destroyed during cloning:	No
5' Sequencing primer:	CMV-F (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	Unknown
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
Sequence:	View sequence
Author's Map:	View map
Plasmid Provided In:	DH5a
Principal Investigator:	Marc Montminy
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result

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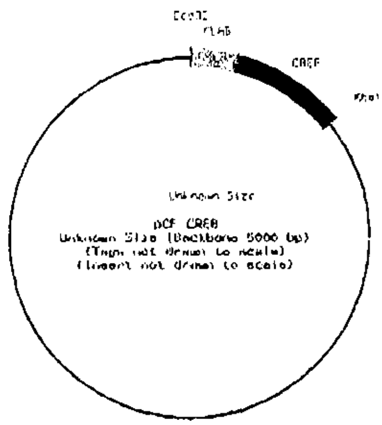
- Nina Papavasiliou Lab Plasmids
- More



Article: Characterization of a CREB gain-of-function mutant with constitutive transcriptional activity in vivo. Du K et al. (Mol Cell Biol. 2000 Jun . 20(12):4320-7. Pubmed)

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

Also, please include the text "Addgene plasmid 22968" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 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 ~~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, 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: DR. MARC TINI
SIGNATURE: [signature]
DEPARTMENT: PHYSIOLOGY AND PHARMACOLOGY
ADDRESS: SIEBENS-DRAKE MEDICAL RES. INSTITUTE, RM 237
PHONE NUMBER: 850-2942
EMERGENCY PHONE NUMBER(S): (519) 694-9102
EMAIL: MTINI@UWO.CA

Location of experimental work to be carried out: Building(s) SDMRI Room(s) 237, 238

*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: CANADIAN CANCER SOCIETY
GRANT TITLE(S): MOLECULAR ANALYSIS OF NOVEL MECHANISMS REGULATING MAMMALIAN GENOME STABILITY AND GENE EXPRESSION

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:

RYAN MCMAN
DEBBIE DONG

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 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	PHAC or CFIA Containment Level
1.	REPLICATION DEFECTIVE	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.010L 1X10^6/mL	PRODUCED IN LAB	○ 1 ☒ 2 ○ 3
	MALONEY MURINE LEUKEMIA VIRUS	○ Yes ☒ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
2.	LABORATORY ESCHERICHIA COLI	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.5L 1X10^9 cfu/mL	PROMEGA	☒ 1 ○ 2 ○ 3
3.	BACULOVIRUS (INSECT VIRUS)	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.050L 3X10^7 pfu/mL	PRODUCED IN LAB	☒ 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		Not applicable
Rodent	☒ Yes ☒ No	MOUSE EMBRYO FIBROBLASTS	2006-056-08
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ No		

* 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 the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	HELA, HELAS3, MCF7	ATCC
Rodent	☒ Yes ○ No	NIH3T3	ATCC
Non-human primate	☒ Yes ○ No	COS7	ATCC
Other (specify)	☒ Yes ○ No	SF9 (INSECT)	ATCC

*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

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		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)	BRAIN TUMOR TISSUE BANK	○ Yes ☒ No		☒ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)	ONTARIO TUMOR BANK	○ 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
DH5X, BL21 E. Coli	PCMX, PET PCMX, PGEX	CLONTECH NOVAGEN GE LIFE SCIENCES	THYMINE DNA GLYCOSYLASE AND ASSOCIATED TRANSCRIPTION FACTORS	POTENTIAL CYTOTOXICITY DUE TO EXPRESSION OF MAMMALIAN PROTEIN

* 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
MALONEY MURINE LEUKEMIA VIRUS	PLNCX	CLONTECH	THYMINE DNA GLYCOSYLASE	POTENTIAL CELL CYCLE AND DNA REPAIR DEFECTS

* Please attach a Material Safety Data Sheet or equivalent.

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 ☒ NO
- E1A oncogene ○ YES ☒ 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 ____________

6.3 AUS protocol # ____________

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(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? ○ 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? ○ Initiate and maintain colony, give location: ________
○ "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 X 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 X NO

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _________ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained

SIGNATURE ______________________________

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

13.0 Containment Levels

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

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

X YES, permit # if on-campus BIO-UWO-0151

O NO, please certify

O NOT REQUIRED for Level 1 containment

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 [signature] Date: May 11/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: [signature] Date: 30 June 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: ______ Date: ______

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: [signature] Date: June 26/09

Approval Number: BIO-UWO-0151 Expiry Date (3 years from Approval): June 25, 2012

Special Conditions of Approval:

- See attached Viral Vector Policy

Policy on Research Utilizing Virus Vector Transduced Cells or Virus Infection of Animals

Version 5

For Biohazards Subcommittee: May, 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 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 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.

Subject: RE: Biohazardous Agents Registry Form: Tini
From: Marc Tini <mtini@uwo.ca>
Date: Tue, 26 May 2009 15:46:30 -0400
To: 'Jennifer Stanley' <jstanle2@uwo.ca>

Hello Jennifer,

In Section 7.1 I am referring to fetal bovine serum used in cell culture media.
We do not perform any pseudotyping of virus. We produce ecotrophic and amphotropic virus using the Phoenix packaging cell lines (http://www.stanford.edu/group/nolan/tutorials/retpkg_7_phx_sys.html).

Regards,
Marc

Marc Tini, PhD
Department of Physiology and Pharmacology
Siebens-Drake Medical Research Institute, Rm. 237
Schulich School of Medicine and Dentistry
University of Western Ontario
London, Ontario N6G 2V4
Tel: Office voice/fax (519) 850-2942,
Lab (519) 661-2111 ext 31321
Fax: (519) 661-3827
http://publish.uwo.ca/~mtini/

-----Original Message-----
From: Jennifer Stanley (mailto:jstanle2@uwo.ca)
Sent: May 26, 2009 3:03 PM
To: Marc Tini
Subject: Biohazardous Agents Registry Form: Tini

Dr. Tini
The Biohazards Subcommittee recently reviewed your project (see attached).
Please let us know the following questions:
1. What is the goat/sheep/cattle product you use in Section 7.1?
2. Is there any pseudotyping of virus done or is it ecotropic?
Thanks
Jennifer

In vertebrate genomes the cytosine-guanine (CpG) dinucleotides mutate at a high rate due to spontaneous deamination of cytosine which results in CpGs occurring at only one quarter of the expected frequency. The CpG mutation rate is increased by methylation of cytosine, which promotes deamination to form thymine. Consequently, in humans the incidence of 5-methylcytotosine to thymine mutations is 10 to 50-fold higher than other base transitions. As a result, more than one third of germline point mutations in genetic diseases and a large fraction of somatic mutations in cancer are due to CpG hypermutability. Cytosine methylation also has an integral role in transcriptional regulation and the promoter regions of genes are enriched in CpG. The integrity of CpGs in these regions is safeguarded by thymine DNA glycosylase which removes aberrant thymine and uracil from G:T and G:U mispairs produced by deamination of 5-methylcytosine and cytosine, respectively. TDG also functionally interacts with transcription factors and epigenetic regulators and serves as a transcriptional cofactor. In previous studies, we have shown that TDG associates directly with protein acetylases CBP (CREB-binding protein) and p300, and the resulting complexes participate in both DNA repair and transcriptional regulation. TDG strongly activates CBP/p300-dependent transcription and reciprocally serves as a substrate for CBP/p300 acetylation. We have shown that two Small Ubiquitin-like Modifier-1 (SUMO-1) protein-binding motifs in TDG are essential for transcriptional stimulation and regulation of subnuclear localization. Moreover, sumoylation of TDG abrogates DNA binding and CBP/p300 association. Recently, our studies have revealed that protein kinase C α (PKCα) interacts with the amino-terminal region of TDG and phosphorylates serine residues adjacent to lysines acetylated by CBP/p300. We found that acetylation and phosphorylation are mutually exclusive and their interplay dramatically alters the DNA mispair processing functions. Remarkably, acetylation selectively prevents G:T processing by abrogating DNA interactions. In contrast, phosphorylation does not alter substrate interactions but may preserve G:T processing *in vivo* by preventing CBP-mediated acetylation. These findings highlight the importance of posttranslational modifications (PTMs) in regulating TDG and suggest that their interplay *in vivo* is critically important in CpG maintenance and epigenetic regulation. Consistent with the important roles of TDG, we have demonstrated that disruption of the *Tdg* gene causes embryonic lethality in mice. In view of the instability of CpG dinucleotides, and the link between aberrant genomic methylation and cancer, elucidation of the physiological roles and regulation of TDG will further our understanding of the molecular processes deregulated in cancer cells. I plan to further investigate the biological functions of TDG by pursuing the following objectives.

Aim I. Molecular analysis of the genome maintenance and gene regulatory functions of TDG: To further elucidate the genome surveillance and transcriptional roles of TDG, we will map the *in vivo* binding sites in the entire genome using chromatin immunoprecipitation on chip (ChIP-chip) assays. We will also use ChIP assays to decipher the mechanisms that target TDG to promoters and determine the role of PTMs in regulating the CpG maintenance and transcriptional functions.

Aim II. Characterization of TDG posttranslational modifications in normal and transformed cells: We will employ PTM-specific antibodies and 2-D polyacrylamide gel electrophoresis to investigate the physiological context for these modifications and establish whether alterations in PTMs are associated with different types of human cancer.

Aim III. Elucidation of the developmental and physiological roles of TDG: We will continue our analysis of *Tdg* null mice and identify potential causes of embryonic lethality by characterizing tissue, cellular and gene expression defects. Additionally, to permit the analysis of cancer susceptibility resulting from TDG deficiency, we will generate mice with a conditional *Tdg* allele that can be ablated in a temporal spatial specific manner.

These investigations will contribute to a better understanding of the molecular events that regulate genome stability and should uncover new molecular tools for the diagnostic and therapeutic management of cancer