Modification Form for Permit BIO UWO 0218

Permit Holder: Andrew Leask

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

Matthew McCann

Kun Huh

Sunil Parapuram

Mohit Kapoor

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	DH5 alpha	Omeslink Feline lentivirus www.genecopoeia.com/product/lentiviral/
Approved Cells	human (primary): foreskin, rodent (primary): mouse sla-, rodent (established): MH 3T3. HEK 293T	
Approved Use of Human Source Material		
Approved GMO	pTEN	
Approved use of Animals	Mouse	

* *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.*

Date of last Biohazardous Agents Registry Form Aug 29, 2008

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-02118

Permit Holder: Andrew Leask

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.*

Date of last Biohazardous Agents Registry Form Aug 29, 2008

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

-------- Original Message --------
Subject: Re: Leask Project involving Feline Lentivirus
Date: Fri, 07 Aug 2009 15:40:37 -0400
From: Andrew Leask <Andrew.Leask@schulich.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
References:<4A7C591D.8000901@uwo.ca>

Dear Jennifer:

Thanks for the clarification; it is easy, then!

PTEN is a tumor suppressor, so overexpressing it wound not cause tumorigenesis; it would suppress tumorigenesis.

See below

http://ghr.nlm.nih.gov/gene=pten

"What is the normal function of the PTEN gene?
The PTEN gene provides instructions for making a protein that is found in almost all tissues in the body. This protein acts as a tumor suppressor, which means that it helps regulate the cycle of cell division by keeping cells from growing and dividing too rapidly or in an uncontrolled way....The PTEN enzyme acts as part of a chemical pathway that signals cells to stop dividing and triggers cells to undergo a form of programmed cell death called apoptosis. These functions prevent uncontrolled cell growth that can lead to the formation of tumors."

So, I think based on this, it should be a Level 2.

Andrew

>>> Jennifer Stanley <jstanle2@uwo.ca> 8/7/2009 12:41 PM >>>
Dr. Leask

I re-looked at the offical Committee's minutes, it states:

Based on revised viral vector policy, it is Level 2 plus unless researcher can demonstrate overexpression of PTEN does not cause tumourgenesis.**

Can you clarify this? Sorry that I missed this!

Jennifer

-------- Original Message --------
Subject: Re: Containment Level request: feline lentiviral vector
Date: Wed, 21 Jan 2009 15:15:47 -0500
From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Dear Ms. Stanley,

Based on the Human Pathogens Importation Regulations, to import this material you would need a containment level 2 permit for HEK 293 cells and a notice for a purified lentivirus vector.

Now, the actual containment and operational procedures related to the laboratory work done with this material is under your responsibility. Recombinant material risk assessments are usually more complexed considering that we are dealing with recombinant material. I can assist you with this risk assessment.

As a start HEK 293 cells alone are classified risk group 2 pathogens.

Lentiviral work is normally conducted in a containment level 2 physical laboratory with the addition of containment level 3 operational practices. However, feline immunodeficiency virus is a Risk Group 2 animal pathogens, so you might decide that containment level 2 physical and operational procedures are sufficient.

You also need to evaluate the risk of the transgene. Is PTEN an oncogene? Does it affect the cell cycle? Can it increase the risk group?

Also consider the work intent? Is it large scale or laboratory scale work? Will the infection be carried in vitro or in vivo? Which species and kind of cells are targeted by the pseudovirus?

You need to evaluate all these parameters and their risk levels in order to determine the physical and operational requirements. The lowest containment level requirements for this particular workdue to the HEK 293 cells is containment level 2 (physical and operational). You might evaluate that containment level 2 physical laboratory with the addition of containment level 3 operational practices or that containment level 3 (physical and operational) is more suitable. Personally, and this is based only on the information available to me at the moment, I would most probably go for containment level 2 physical laboratory with the addition of containment level 3 operational practices. But again, you have the final word and ultimately it is your responsibility.

I hope this information is clear enough. Do not hesitate to contact me if you need further assistance with this risk assessment.

Regards

Genevieve Lacroix
A/Head, Importation and Biosafety Program/
Chef Intérimaire, Importation et Services de biosécurité
Office of Laboratory Security / Bureau de la 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) 946-6982

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

Subject: Re: Biohazardous Agents Registry Form Modification - Dr. Leask - FelineLentivirus
Date: Wed, 10 Dec 2008 14:50:06 -0500
From: Andrew Leask <Andrew.Leask@schulich.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
References: <49401091.3030207@uwo.ca>
<493FD581.647C.0067.0@schulich.uwo.ca>
<49401C56.5000404@uwo.ca>

http://www.safety.rochester.edu/ibc/ibcvirus.htm

The above is a safety committee reference. I will send the other information as soon as I get it

>>> Jennifer Stanley <jstanle2@uwo.ca> 12/10/2008 2:45 PM >>>
Fabulous, thanks

Andrew Leask wrote:
> Dear Jennifer:
> Thank you for this note. I will be sending you a separate email (a brochure) that confirms that the virus is feline based.
>
> We wish to use the 293 cells as packaging cells to grow up a feline virus which will allow the overexpression of PTEN in primary rodent cells.
>
> I will send you the fax shortly.
> best wishes
>

PTEN (gene)

From Wikipedia, the free encyclopedia

edit

In the field of molecular biology, **phosphatase and tensin homolog** also known as **PTEN** is a protein which in humans is encoded by the *PTEN* gene.[2] *PTEN* acts as a tumor suppressor gene through the action of its phosphatase protein product. This phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly. Mutations of this gene contribute to the development of certain cancers.[3]

This gene was identified as a tumor suppressor that is mutated in a large number of cancers at high frequency. The protein encoded this gene is a phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase. It contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, this protein preferentially dephosphorylates phosphoinositide substrates. It negatively regulates intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells and functions as a tumor suppressor by negatively regulating Akt/PKB signaling pathway.[4]

Contents

Function and structure

The corresponding PTEN protein is found in almost all tissues in the body. PTEN protein acts as a phosphatase to dephosphorylate phosphatidylinositol (3,4,5)-trisphosphate (PtdIns (3,4,5)P_3 or PIP_3). PTEN specifically catalyses the dephosporylation of the 3` phosphate of the inositol ring in PIP_3, resulting in the biphosphate product PIP_2 (PtdIns(4,5) P2). This dephosphorylation is important because it results in inhibition of the AKT signaling pathway.

Phosphatase and tensin homolog (mutated in multiple advanced cancers 1)

Crystallographic structure of human PTEN. The N-terminal phosphatase domain is colored blue while the C-terminal C2 domain is colored red.[1]

Available structures: 1d5r

Identifiers

Symbols	PTEN; BZS; MGC11227; MHAM; MMAC1; PTEN1; TEP1
External IDs	OMIM: 601728 MGI: 109583 HomoloGene: 265
EC number	3.1.3.67

Gene ontology [show]

Orthologs

	Human	Mouse
Entrez	5728	19211
Ensembl	ENSG00000171862	ENSMUSG00000013663
Uniprot	P60484	Q3UFB0
Refseq	NM_000314 (mRNA) NP_000305 (protein)	NM_008960 (mRNA) NP_032986 (protein)
Location	Chr 10: 89.61 - 89.72 Mb	Chr 19: 32.82 - 32.89 Mb
Pubmed search	[1]	[2]

The structure of PTEN (solved by X-ray crystallography, see figure to the upper right[1]) reveals that it

consists of a phosphatase domain, and a C2 domain: the phosphatase domain contains the active site which carries out the enzymatic function of the protein, whilst the C2 domain binds the phospholipid membrane. Thus PTEN binds the membrane through its C2 domain bringing the active site to the membrane-bound PIP_3 to de-phosphorylate it.

When the PTEN enzyme is functioning properly, it acts as part of a chemical pathway that signals cells to stop dividing and causes cells to undergo programmed cell death (apoptosis) when necessary. These functions prevent uncontrolled cell growth that can lead to the formation of tumors. There is also evidence that the protein made by the PTEN gene may play a role in cell movement (migration) and sticking (adhesion) of cells to surrounding tissues.

Clinical significance

PTEN is one of the most commonly lost tumour suppressors in human cancer. During tumor development, mutations and deletions of PTEN occur that inactivate its enzymatic activity leading to increased cell proliferation and reduced cell death. Frequent genetic inactivation of PTEN occurs in glioblastoma, endometrial cancer, prostate cancer, and reduced expression is found in many other tumor types such as lung and breast cancer.

PTEN mutation also causes a variety of inherited predispositions to cancer.

Cowden syndrome: Researchers have found more than 70 mutations in the PTEN gene in people with Cowden syndrome. These mutations can be changes in a small number of base pairs or, in some cases, deletions of a large number of base pairs. Most of these mutations cause the PTEN gene to make a protein that does not function properly or does not work at all. The defective protein is unable to stop cell division or signal abnormal cells to die, which can lead to tumor growth, particularly in the breast, thyroid or uterus.

Other disorders: Mutations in the PTEN gene cause several other disorders that, like Cowden syndrome, are characterized by the development of noncancerous tumors called hamartomas. These disorders include Bannayan-Riley-Ruvalcaba syndrome, Proteus syndrome, and Proteus-like syndrome. Together, the disorders caused by PTEN mutations are called PTEN hamartoma tumor syndromes, or PHTS. Mutations responsible for these syndromes cause the resulting protein to be nonfunctional or absent. The defective protein allows the cell to divide in an uncontrolled way and prevents damaged cells from dying, which can lead to the growth of tumors.

See also

- Multiple hamartoma syndrome

References

1. ^ *a b* PDB 1d5r; Lee JO, Yang H, Georgescu MM, Di Cristofano A, Maehama T, Shi Y, Dixon JE, Pandolfi P, Pavletich NP (October 1999). "Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association". *Cell* **99** (3): 323–34. doi:10.1016/S0092-8674(00)81663-3. PMID 10555148.
2. ^ Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Swedlund B, Teng DH, Tavtigian SV (April 1997). "Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers".

Nat. Genet. **15** (4): 356–62. doi:10.1038/ng0497-356. PMID 9090379.
3. ^ Chu EC, Tarnawski AS (October 2004). "PTEN regulatory functions in tumor suppression and cell biology". *Med. Sci. Monit.* **10** (10): RA235–41. PMID 15448614. http://www.medscimonit.com/fulltxt.php?ICID=11797.
4. ^ "Entrez Gene: PTEN phosphatase and tensin homolog (mutated in multiple advanced cancers 1)".

Further reading

- Li J, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R (1997). "PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer.". *Science* **275** (5308): 1943–1947. doi:10.1126/science.275.5308.1943. PMID 9072974.
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- Pilarski R, Eng C (2004). "Will the real Cowden syndrome please stand up (again)? Expanding mutational mutational and clinical spectra of the PTEN hamartoma tumour syndrome". *J Med Genet* **41** (5): 323–6. doi:10.1136/jmg.2004.018036. PMID 15121767.
- Sansal I, Sellers WR (2004). "The biology and clinical relevance of the PTEN tumor suppressor pathway". pathway". *J Clin Oncol* **22** (14): 2954–63. doi:10.1200/JCO.2004.02.141. PMID 15254063.
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- Zhou XP, Waite KA, Pilarski R, Hampel H, Fernandez MJ, Bos C, Dasouki M, Feldman GL, Greenberg LA, Ivanovich J, Matloff E, Patterson A, Pierpont ME, Russo D, Nassif NT, Eng C (2003). "Germline PTEN promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the phosphoinositol-3-kinase/Akt pathway". *Am J Hum Genet* **73** (2): 404–11. doi:10.1086/377109. PMID 12844284.
- Ji S-P, Zhang Y, Cleemput JV, Jiang W, Liao M, Li L, Wan Q, Backstrom JR, Zhang X (2006). (2006). "Disruption of PTEN coupling with 5-HT2C receptors suppresses behavioral responses induced by drugs of abuse". *Nature Medicine* **12** (3): 324–9. doi:10.1038/nm1349. PMID 16474401.

External links

- The PTEN Protein
- GeneCard
- UMich Orientation of Proteins in Membranes *protein/pdbid-1d5r*
- MeSH *PTEN+Protein*
- Alzgene

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GeneCopoeia™
Expressway to Discovery

OmicsLink™ Human and Mouse Lentiviral ORF Expression Clones

User's Manual # LV001
Version: 08040902

GeneCopoeia, Inc.
19520 Amaranth Drive
Germantown, MD 20874
USA

www.genecopoeia.com

I. Introduction

Currently GeneCopoeia has multiple sets of 20,000 human and 15,000 mouse lentiviral ORF expression clones in several lentiviral expression vectors. The FIV (feline leukemia virus) based vectors are considered biologically safe, yet have been shown to be as effective as HIV based vectors at transduction of genes into a wide variety of dividing and non-dividing mammalian cells. Once cells are infected with the pseudoviral particles, the expression construct integrates with the genome in target cells and is stably expressed.

Advantages of OmicsLink™ Lentiviral Human and Mouse ORF Expression Clones

1. High Efficiency of Gene Delivery to Virtually All Cell Types and Whole Model Organisms
2. High Expression Levels of Delivered Genes
3. Self-inactivation and No Unwanted Viral Replication

II. OmicsLink™ Human and Mouse Lentiviral ORF Expression Clone Types

OmicsLink™ Lentiviral Human and Mouse ORF Expression Clones are offered in the following vector types:

Vector	Promoter	Selection Marker	Tag
pReceiver-Lv01	Lenti-CMV	No	None
pReceiver-Lv41	Lenti-EF1a	Neomycin	None
pReceiver-Lv21	Lenti-CMV	Neomycin	None
pReceiver-Lv26	Lenti-CMV	Neomycin	N-Avi
pReceiver-Lv10	Lenti-CMV	Neomycin	C-Avi
pReceiver-Lv35	Lenti-CMV	No	N-Avi + IRES-Biotin ligase
pReceiver-Lv34	Lenti-CMV	Neomycin	N-eCFP
pReceiver-Lv61	Lenti-CMV	No	C-eCFP
pReceiver-Lv62	Lenti-CMV	Neomycin	C-eCFP
pReceiver-Lv19	Lenti-CMV	Neomycin	N-eGFP
pReceiver-Lv04	Lenti-CMV	No	C-eGFP
pReceiver-Lv08	Lenti-CMV	Neomycin	C-eGFP
pReceiver-Lv20	Lenti-CMV	Neomycin	N-eYFP
pReceiver-Lv05	Lenti-CMV	No	C-eYFP
pReceiver-Lv09	Lenti-CMV	Neomycin	C-eYFP
pReceiver-Lv23	Lenti-CMV	Neomycin	N-Flag
pReceiver-Lv03	Lenti-CMV	No	C-Flag
pReceiver-Lv07	Lenti-CMV	Neomycin	C-Flag
pReceiver-Lv33	Lenti-CMV	No	C-Flag + IRES-eGFP
pReceiver-Lv02	Lenti-CMV	No	C-HA
pReceiver-Lv06	Lenti-CMV	Neomycin	C-HA
pReceiver-Lv32	Lenti-CMV	No	C-HA + IRES-eGFP
pReceiver-Lv64	Lenti-CMV	Neomycin	N-HaloTag
pReceiver-Lv65	Lenti-CMV	Neomycin	C-HaloTag
pReceiver-Lv31	Lenti-CMV	No	IRES-eGFP
pReceiver-Lv36	Lenti-CMV	No	IRES-luciferase
pReceiver-Lv40	Lenti-CMV	Neomycin	IRES-Neomycin
pReceiver-Lv25	Lenti-CMV	Neomycin	N-Myc
pReceiver-Lv17	Lenti-CMV	Neomycin	C-Myc
pReceiver-Lv43	Lenti-CMV	No	C-Myc+ IRES-eGFP
pReceiver-Lv44	Lenti-CMV	No	C-Myc+ IRES-eYFP
pReceiver-Lv47	Lenti-CMV	Neomycin	C-Myc+IRES-Neomycin
pReceiver-Lv18	Lenti-CMV	Neomycin	C-Myc-His

III. Getting Started.

We recommend on receipt of a new expression clone that the plasmid is transformed into DH5α or equivalent cells (the expression plasmid contains the ampicillin resistance gene) and that a midi-prep of the plasmid is made. As the efficiency of transfection is dependent on the quality of plasmid DNA, we recommend the use of the QIAGEN Endotoxin-free Plasmid Kit or purification by CsCl gradient. Normal transfections use 2ug of plasmid for each 10-cm plate.

IV. Pseudovirus Production

The lentiviral expression vector contains the elements required for packaging, transduction, and stable integration of the viral expression construct into genomic DNA, leading to expression of the open reading frame. However, it lacks the elements essential for transcription and packaging of an RNA copy of the expression construct into recombinant pseudoviral particles. These factors are supplied by a packaging kit (Cat. # pLV-PK-01) that comes with a mixture of two packaging plasmids, pLV-PK-FIV and pLV-PK-VSG. pLV-PK-FIV contains the structural, regulatory, and replication genes required to produce lentivirus. pLV-PK-VSG expresses the envelope protein of vesicular stomatitis virus, which defines the range of infectible cells. The packaging kit, pLV-PK-01, also has a positive lentiviral expression clone that expresses eGFP protein.

To generate pseudoviral particles, producer cells need to be transiently co-transfected with the expression clone and packaging vectors. After transfection, the producer cells secrete pseudoviral particles into the culture media. The pseudoviral particles can be used to directly infect target cells. The expression construct is reverse transcribed and integrated into the genome of target cells, providing high levels of expression of the open reading frame of choice. See figure 1 for schematics of this process.

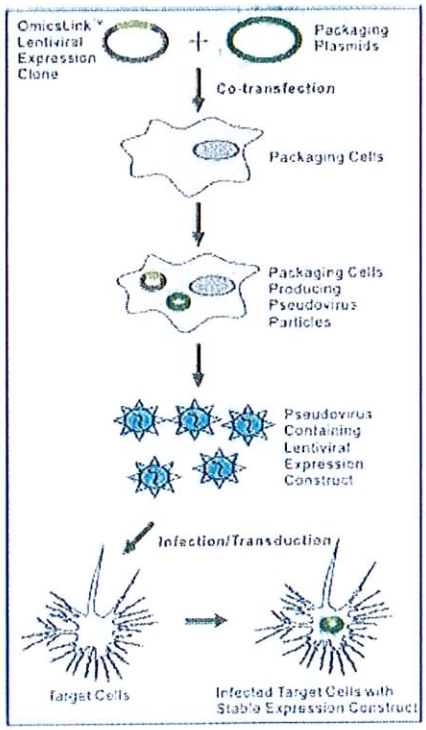

Figure 1. Schematics of pseudovirus production and infection of target cells

The following protocol describes the generation of pseudoviral particles containing the lentiviral expression construct. The yield of recombinant lentiviral particles typically produced under these optimized conditions is 10 ml of 1-3x10^6 ifu/ml for the FIV system per 10-cm culture plate when measured by transduction of H1299 cells. This amount of pseudoviral particles is generally sufficient to infect 5-10x10^5 target cells at a MOI (multiplicity of infection) equal to 1.

Caution: Following this protocol results in the production of infectious pseudoviral particles. The recommended guidelines for working with BSL-2 safety class must be adhered to.

1. We recommend the use of 293Ta as producer cells. These should be grown in D-MEM medium supplemented with heat-inactivated serum and antibiotics for 2 to 3 days (50-70% confluency) prior

to transfection.

2. Mix 10 µg of the packaging plasmid mix with 2µg of the lentiviral expression construct (eGFP control plasmid at a 1:100 ratio may be included at this point if desired). Add the mixture to 400 µl D-MEM medium without serum and antibiotics. Add 20 µl of Plus™ Reagent (Invitrogen, Cat. # 11514-015), mix, and incubate at room temperature for 15 min.
3. Dilute 30 µl of Lipofectamine™ Reagent (Invitrogen, Cat. # 18324-111) into 400 µl of D-MEM medium without serum and antibiotics. Mix gently.
4. Add diluted Lipofectamine™ Reagent (from step 3) dropwise to DNA/Plus™ Reagent complex (from step 2). Mix gently by inversion and incubate at room temperature for 15min.
5. While incubating the mixture in step 4 to form the transfection complexes, wash the 293Ta cells with 10 ml of D-MEM without serum and antibiotics, and then add 9 ml of D-MEM with 2% serum and without antibiotics.
6. Add the DNA/ Plus™ Reagent/ Lipofectamine™ Reagent complex (from step 4) to the plate from step 5, and mix complexes with medium gently by inversion and incubate at 37°C in a CO_2 incubator overnight.
7. Replace the medium containing complexes with fresh D-MEM medium supplemented with 2% serum and antibiotics and continue incubation in the CO_2 incubator at 37°C . Peak virus production is normally achieved 48 hours post infection. We recommend collecting supernatants at 24, 36, 48, and 60 hours. Supernatants should be replaced with fresh D-MEM medium supplemented with 2% serum without antibiotics.
8. Collect all 10 ml of the pseudovirus-containing medium in a 15-ml sterile capped conical tube, and centrifuge at 3,000 rpm at room temperature for 5 minutes to pellet cell debris.
9. Following centrifugation, filter the supernatant through Millex-HV 0.45 µm PVDF filters (a low protein binding filter from Millipore, Cat. #SLHVR25LS).

The supernatant containing the pseudoviral particles can be used directly to determine the pseudoviral titer and directly used to infect target cells *in vitro* as long as the target cells can survive in conditioned medium. The supernatant can be stored at –80°C without cryoprotectant. Expect a 20-30% loss of viral titer with each freeze/thaw cycle.

V. Pseudoviral Titer Estimation

At this point we recommend that the pseudoviral stock is titered to ensure it is viable and to test what fraction of target calls can be transduced. This enables the number of copies of viral construct per target cell to be controlled.

Pseudoviral titer is normally achieved by mixing the eGFP control plasmid at a 1:100 ratio with the expression construct as an internal control. Alternatively the eGFP control plasmid can be expressed in parallel as an external control. To determine the relative pseudoviral titer, the packaged lentiviral expression clone should be transduced into H1299 cells along with cell lines appropriate to the user's experimental system. Relative titers may vary up to 20 fold.

Day 1.

1. For each viral stock, plate H1299 cells in a 24-well plate at a density of $0.6\text{-}1x10^5$ cells per well 24 hours prior to viral infection. Add 1 ml of complete D-MEM medium (with serum and antibiotics) and incubate cells at 37°C with 5% CO_2 overnight.

Day 2.

2. Prepare complete D-MEM medium plus 10% FBS with Polybrene at a final concentration of 5µg/ml. (The optimal concentration of Polybrene depends on cell type and may need to be empirically determined, but is usually in the range of 2-10 µg/ml). Prepare enough for an extra well as a negative control.

3. Remove culture medium and replace with 0.5 ml of complete D-MEM medium with 10% serum and Polybrene (from Step 2). For each pseudoviral stock, use three wells. Infect H1299 cells by adding 1 µl of viral stock into the first well (dilution factor of 500), 10 µl of viral stock into the second well (dilution factor of 50), and 100 µl of viral stock into the third well (dilution factor of 5). For one additional well (mock well control), add 0.5ml of D-MEM medium with Polybrene (from Step 2). Incubate cells at 37°C with 5% CO_2

overnight. (Note: Excessive exposure to Polybrene (>12 hr) can be toxic to some cells.)

Day 3.

4. Remove culture medium and replace with 1 ml of complete D-MEM medium (without Polybrene). Incubate the cells at 37°C with 5% CO_2 overnight.

Day 4.

5. Split the cells 1:3 to 1:5, depending on the type of cells, and incubate in complete D-MEM for an additional 24 hours.

Day 6.

6. The fraction of eGFP fluorescent cells can be counted by FACS (fluorescent activated cell sorting). Alternatively the eGFP fluorescence may be visualized. Normally 10 random fields of view are used to estimate the overall fraction of fluorescing cells on a plate. The average of this number is multiplied by $1.5x10^5$ (expected # of cells on plate at the time of infection) and by the corresponding dilution factor, then divide by 0.5ml to determine the relative titer of the pseudovirus in the supernatant.

VI. Transduction of the Packaged Lentiviral Expression Clones

The transduction efficiency depends upon the target cells and experimental procedure. It is recommended that the titered pseudoviral stock containing the positive control eGFP construct is used to determine the concentration of pseudoviral particles required for the desired MOI of target cells. After these test transductions are performed, it should be possible to determine the optimum concentration of pseudoviral particles for transduction based on eGFP fluorescence.

Day 1.

1. Plate target cells in a 24-well plate at a density of $0.6\text{-}1x10^5$ cells per well 24 hours prior to viral infection. Add 1 ml of complete optimal medium (with serum and antibiotics) and incubate cells at 37°C with 5% CO_2 overnight.

Day 2.

2. For each well, prepare 0.5 ml of virus suspension diluted in complete medium with Polybrene at a final concentration of 5-8 μg/ml.

Note:

a. We recommend to start with 0.5 ml of non-diluted viral supernatant (if the virus was not concentrated), if possible. The higher the concentration of virus in solution, the higher is the transduction efficiency.
b. Use several dilutions of pseudoviral stock if necessary. In addition, we recommend including a transduction with the eGFP control construct and other appropriate positive and negative control constructs.
c. Mix the virus with the medium gently by rotation or inversion. Do not vortex.

3. Infect target cells by removing the culture medium from cells and replacing it with 0.5 ml of prepared virus dilution. For one well (mock well control), add 0.5 ml of D-MEM medium with Polybrene. Incubate cells at 37°C with 5% CO_2 overnight. For extremely fast-growing and metabolizing cell lines such as 293Ta, use 3% FBS in the medium.

Day 3.

4. Remove the culture medium and replace with 1 ml of complete medium (without Polybrene). Incubate the cells at 37°C with 5% CO_2 overnight.

Day 4.

5. By day 4, the culture will be confluent (depending on cell type). Split it 1:3 to 1:5, depending on the type of cells, and continue incubating for 48 hours in complete D-MEM.

Day 6.

6. The infected target cells can be analyzed for transient expression of the expression construct using an appropriate biological assay. If you have used an internal eGFP control, determine the percentage of infected cells by counting fluorescing cells by flow cytometry. Alternatively, the infected cells can be identified by selecting and counting based on selection marker genes contained in the expression clone constructs (*e.g.*, neomycin), but the titer determined by neomycin selection is usually less than the titer determined by eGFP selection.

VII. Limited Use License and Warranty

GeneCopoeia, Inc.
19520 Amaranth Drive
Germantown, MD 20874
Tel: 301-515-6982
Fax: 301-515-6983
Email: inquiry@genecopoeia.com
Web: www.genecopoeia.com

PHYSIOLOGY 473B/573B: Cell Signaling in Tissue, Injury & Repair
[old name: Advanced Cellular Physiology]

Course Manager: Dr. R. Wang

Wednesday, 1:30-3:30, Jan. 7 -- April 8, 2009
Medical Sciences Building, Room 282

INSTRUCTORS:
Instructors:
Dr. R. Wang, VRL,Rm A5-140, 685-8500 x55098 [on Sabbatical Leave]
Dr. A. Leask, *DSB 0067, 661-2111 x81102* [Course Manager]
Dr. S. Cregan , Robarts Research Institute, Rm 308, 663-5777 x34134

LECTURE SCHEDULE:

Date	Lecturer	Topic
January 7	Cregan	Molecular mechanisms of proliferation and differentiation in neurodevelopment
January 14	Cregan	Molecular mechanisms of apoptosis
January 21	Cregan	Non-apoptotic mechanisms of cell death [In Class Term Exam 1hr]
January 28	Cregan	Cell death in neurodevelopment and neurodegenerative conditions
February 4	Leask	Cell adhesion, contraction and matrix deposition in normal tissue repair
February 11	Leask	Signaling in fibrotic disease (I)
February 18	Leask	Signaling in fibrotic disease (II) [In Class Midterm - 1hr]
Feb. 23-March 1	CLASS CANCELLED - STUDY WEEK	
March 4	Leask	Function and regulation of the CCN family of proteins
March 11	Student Presentations	
March 18	Student Presentations	
March 25	Student Presentations	
April 1	Student Presentations	
April 8	Student Presentations	
Last day of class: April 10, 2009 **Final Examination Period: Apr. 10 - 30, 2009 (2hr)**		

General Information About Mammalian Virus Vectors

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All use of **mammalian** recombinant virus vectors at UR must be approved by the IBC through the registration process. *Non-mammalian vectors (such as baculovirus) are exempt from this requirement.*

Please note that the NIH Guidelines stipulate that Institutional Biosafety Committee approval be obtained prior to use of recombinant mammalian virus vector systems. See NIH Guidelines Section III-D.

Overview of selected vector systems

General comment on containment: Suggested biosafety containment levels are provided for each vector system. Note, however, that use of a higher-level containment facility may be required in some cases, depending on the specific properties of the vector and/or insert. **Special care** should be given to the design, risk assessment (Section 2 of NIH Guidelines), and handling of virus vectors containing genes that make growth-regulating products, products released into the circulation, products that may have a general effect on the host-immune system.

Adenovirus: Adenoviruses are infectious human viruses, which often cause mild respiratory illness. Rare cases of severe disease can occur, and its use as a genetic vector therefore requires the use of adequate containment equipment and practices. Biosafety Level 2 (BL2) is appropriate for many constructs (see above). **Particular care** should be given to vectors containing genes that make products that may be similar to products made by the deleted adenovirus genes.

See Adenovirus Vector Safety Guidelines for additional information.

Adeno-associated virus: These are infectious human viruses with no known disease association. Some AAV types are common in the general population, and these viruses have the ability to integrate into the host chromosome. The NIH Guidelines (Appendix B) state that "adeno-associated virus (AAV) types 1 through 4, and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus" can in most cases be handled at biosafety level 1 (BL1). This level of containment made is modified by other considerations (see above).

Herpesvirus: Herpesviruses include infectious human viruses such as herpes simplex virus type-1 (HSV-1), which is the most commonly used vector system. HSV-1 is

common in the general population, but can cause encephalitis in rare cases; its utility as a vector system stems from its broad host cell range, ability to transduce neurons, and its large insert capacity. Biosafety Level 2 (BL2) is appropriate for many constructs (see above).

Lentivirus: Lentiviruses are a subset of retroviruses, with the ability to integrate into host chromosomes, and to infect non-dividing cells. These viruses can cause severe immunologic and neurologic disease in their natural hosts. Lentivirus vector systems can include viruses of non-human origin (feline immunodeficiency virus, equine infectious anemia virus) as well as simian viruses (simian immunodeficiency virus) and human viruses (HIV). Typical lentivirus vectors take the form of virus pseudotypes bearing envelope proteins from vesicular stomatitis virus (VSV). It is usually recommended that work with non-human lentiviruses that are incapable of establishing productive infections in humans (FIV, EIAV) be conducted at biosafety level 2 (BL2). Work with simian or human lentiviruses (SIV, HIV) is typically conducted at a higher containment level (please consult the IBC).

> **See FIV Vector Safety Guidelines for additional information.**
>
> **See NIH OBA's Biosafety considerations for Research with Lentiviral vectors for additional information.**
>
> **See Assessment for Recombinant, Pseudotyped Lentiviral Vectors (HIV and SIV vectors) for additional information.**
>
> **See Assessment for Recombinant Amphotropic Retroviral Vectors Containing Oncogenes and Lentiviral Vectors Containing Oncogenes for additional information.**

Poxvirus: Poxvirus vectors include avian viruses (avipox vectors) such as NYVAC and ALVAC, which cannot establish productive infections in humans, as well as mammalian poxviruses, which can productively infect humans -- such as vaccinia virus and modified vaccinia viruses (MVA). Poxviruses are highly stable, and vaccinia virus can (rarely) cause severe infections in immunocompromised persons, persons with certain underlying skin conditions, or pregnant women. Such individuals should not work with vaccinia virus (see IBC guidelines). The use of biosafety Level 2 (BL2) is appropriate for many poxvirus and constructs (see above).

Retrovirus: These are infectious viruses which can integrate into transduced cells with high frequency, and which may have oncogenic potential in their natural hosts. Retrovirus vector systems are typically based on murine viruses -- most commonly, these systems include ecotropic viruses (which can infect only murine cells), amphotropic viruses (which can infect human cells) or pseudotyped viruses (which can also infect human cells). Containment for vectors with the ability to infect human cells will usually be recommended at biosafety level 2 (BL2), as per the NIH Guidelines (Appendix B) *("For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains*

of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended."), whereas for ecotropic vectors with no ability to infect human cells, BL1 containment may be appropriate *("[ecotropic] murine retroviral vectors....that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment").*

Baculovirus: Non-mammalian virus vectors are exempt from these registration requirements.

See Suggested Biocontainment Levels for Common Viral Vectors for additional information.

PDF version: **Mammalian Virus Vector Registration**

Word version: **Mammalian Virus Vector Registration**