Modification Form for Permit BIO-UWO-0019

Permit Holder: Shun-Cheng (Shawn) Li

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

Ran Wei

Marek Galka

Courtney Voss

Gurpreet Dhami

Xuan Cao

Karen Kennedy

Shelly Sandiford

Thamara Dayarathna

Zezhou Wang

Chengjun Li

Additional Personnel
(Please list additional personnel here)

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Oct 10, 2007

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-0019

Permit Holder: Shun-Cheng (Shawn) Li

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E-coli. DH5 alpha, BL21	Lentivirus
Approved Cells	Rodent (primary, spleen, B/T cells), Human (established) BJAB, Jurkat, 293 HEK, LG2, Namalwa, Rodent (established), A20, MeT-5A cell line, A-549 human lung cancer cells	
Approved Use of Human Source Material		
Approved GMO	pMIG, pGEX-4T3, pRc/CMV, pCDNA3.1+/hygro,pDEST15, pGEX-2T, pGEX-4T2, pFLAG/CMV2	pBS-MPM-ALK
Approved use of Animals	mice, rats	
Approved Toxin(s)	Cholera toxin	

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Chair,Biohazards Subcommittee: _____

From: Gurpreet Dhami <g_dhami@yahoo.com>
Date: Thu, 16 Apr 2009 06:52:32 -0700 (PDT)
To: jstanle2@uwo.ca

Dear Jenniffer,

We are not planning to make any dominant negative with lentivirus. All we would like to do is knock down Numb in mammalian cell lines.

Gurpreet

1. Virus used: lentivirus

2. Vectors used: lentiviral vector (pLKO.1)
PMDL, REV AND VSVG are plasmids for packaging of lentivirus

3. Source of vector: give a website for example: Dr. Pierpaolo's lab library.

4. gene transfected: ShRNA against hNumb

5. describe the change that results: Transfection of virus with knockdown hNumb when transfected into mammalian cells

That is the vector which was used to clone Control and Numb ShRNA. Constructs (pLentiLox 3.7 ShRNA ctl and pLentiLox 3.7-ShRNA-Numb) were obtained from Italy

Dr. Pier Paolo Di Fiore
IFOM, the FIRC Institute for Molecular Oncology Foundation,
Via Adamello 16, 20139,
Milan, Italy

That is the company (invitrogen) where we are going to buy Lentivirus from.

NUMB is a cell fate determinant, which, by asymmetrically partitioning at mitosis, controls cell fate choices by antagonising the activity of the plasma membrane receptor of the NOTCH family. NUMB has also been shown to function as an endocytic protein, and the NOTCH-NUMB counteraction has been linked to this function. Recently Numb has been prosposed to regulate the tumor suppressor protein p53 and thus has been proposed to play a role as tumor suppressor in breast cancer.

Gurpreet

Overview, continued

How Lentivirus Works

Once the lentivirus enters the target cell, the viral RNA is reverse-transcribed, actively imported into the nucleus (Lewis & Emerman, 1994; Naldini, 1999), and stably integrated into the host genome (Buchschacher & Wong-Staal, 2000; Luciw, 1996). After the lentiviral construct has integrated into the genome, you may assay for transient expression of your recombinant protein or use antibiotic selection to generate a stable cell line for long-term expression studies.

VSV Envelope Glycoprotein

Most retroviral vectors are limited in their usefulness as gene delivery vehicles by their restricted tropism and generally low titers. In the ViraPower™ Lentiviral Expression System, this limitation has been overcome by use of the G glycoprotein gene from Vesicular Stomatitis Virus (VSV-G) as a pseudotyping envelope, thus allowing production of a high titer lentiviral vector with a significantly broadened host cell range (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).

Biosafety Features of the System

Introduction

The ViraPower™ Lentiviral Expression System is a third-generation system based on lentiviral vectors developed by Dull *et al.*, 1998. This third-generation lentiviral system includes a significant number of safety features designed to enhance its biosafety and to minimize its relation to the wild-type, human HIV-1 virus. These safety features are discussed below.

Biosafety Features of the ViraPower™ Lentiviral System

The ViraPower™ Lentiviral Expression System includes the following key safety features:

- The pLenti expression vector contains a deletion in the 3′ LTR (ΔU3) that does not affect generation of the viral genome in the producer cell line, but results in "self-inactivation" of the lentivirus after transduction of the target cell (Yee *et al.*, 1987; Yu *et al.*, 1986; Zufferey *et al.*, 1998). Once integrated into the transduced target cell, the lentiviral genome is no longer capable of producing packageable viral genome.
- The number of genes from HIV-1 that are used in the system has been reduced to three (*i.e. gag*, *pol*, and *rev*).
- The VSV-G gene from Vesicular Stomatitis Virus is used in place of the HIV-1 envelope (Burns *et al.*, 1993; Emi *et al.*, 1991; Yee *et al.*, 1994).
- Genes encoding the structural and other components required for packaging the viral genome are separated onto four plasmids. All four plasmids have been engineered not to contain any regions of homology with each other to prevent undesirable recombination events that could lead to the generation of a replication-competent virus (Dull *et al.*, 1998).
- Although the three packaging plasmids allow expression *in trans* of proteins required to produce viral progeny (*e.g.* gal, pol, rev, env) in the 293FT producer cell line, none of them contain LTRs or the Ψ packaging sequence. This means that none of the HIV-1 structural genes are actually present in the packaged viral genome, and thus, are never expressed in the transduced target cell. No new replication-competent virus can be produced.
- The lentiviral particles produced in this system are replication-incompetent and only carry the gene of interest. No other viral species are produced.
- Expression of the *gag* and *pol* genes from pLP1 has been rendered Rev-dependent by virtue of the HIV-1 RRE in the gag/pol mRNA transcript. Addition of the RRE prevents gag and pol expression in the absence of Rev (Dull *et al.*, 1998).
- A constitutive promoter (RSV promoter) has been placed upstream of the 5′ LTR in the pLenti expression vector to offset the requirement for Tat in the efficient production of viral RNA (Dull *et al.*, 1998).

continued on next page

Biosafety Features of the System, continued

Biosafety Level 2

Despite the inclusion of the safety features discussed on the previous page, the lentivirus produced with this System can still pose some biohazardous risk since it can transduce primary human cells. For this reason, **we highly recommend that you treat lentiviral stocks generated using this System as Biosafety Level 2 (BL-2) organisms and strictly follow all published BL-2 guidelines with proper waste decontamination.** Furthermore, exercise extra caution when creating lentivirus carrying potential harmful or toxic genes (*e.g.* activated oncogenes).

For more information about the BL-2 guidelines and lentivirus handling, refer to the document, "Biosafety in Microbiological and Biomedical Laboratories," 4th Edition, published by the Centers for Disease Control (CDC). This document may be downloaded at the following address:

http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Important

Handle all lentiviruses in compliance with established institutional guidelines. Since safety requirements for use and handling of lentiviruses may vary at individual institutions, we recommend consulting the health and safety guidelines and/or officers at your institution prior to use of the ViraPower™ Lentiviral Expression System.

Experimental Outline

Flow Chart

The diagram below describes the general steps required to express your gene of interest using the ViraPower™ Lentiviral Expression System. Refer to the appropriate manual for each pLenti expression vector for instructions to generate your pLenti expression construct.