Chambers, Ann F
BIO-LRCC-0022

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.
PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

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

Pieter Anborgh

Nicole Hague

Carl Postenka

Caroline Trieman

David Dales

Additional Personnel
(Please list additional personnel here)

Connor MacMillan
Allen Clifford

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	E. coli DH5alpha, E. coli BL21, E.coli JM109, Lentivirus	
Approved Primary and Established Cells	[Established] (Human): 21T series (21PT, 21NT, 21MT-1) mammary epithelial, breast cancer lines: MDA MB 175, MDA MB 231, MDA MB 231 LucD3H2LN, MDA MB 435, MDA MB 468, MDA MB 468 CON, MDA MB	See Attached Sheet
Approved Use of Human Source Material	[Blood (whole) or other body fluid]: cancer patient whole blood, [Human blood (fraction) or other body fluid]: cancer patient plasma	
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid] pcDNA3.1(+), pcDNA3.1(+)/myc-His B, pZsGreen1-C1, pSuper.puro, pGEX2T, Plko.1-PURO/shRNA, Pglpz/shS100A2, [Vector] pGIPZmir library	
Approved Use of Animals	mouse	
Approved Biological Toxin(s)		

Approved Gene Therapy		
Approved Plants and Insects		

As the Principal Investigator, I have ensured 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.shs.uwo.ca/workplace/newposition.htm

Signature of Permit Holder: Ann D. Cl

Current Classification: 2 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Mar 16, 2012

Date of Last Modification (if applicable):

BioSafety Officer(s)*: Mark Ryder March 22, 2012

***For work being performed at Institutions affiliated with Western University, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Western University Biosafety Officer.**

Chair, Biohazards Subcommittee: **Date:**

MODIFICATION TO BIO-LRCC-0022

Addition of three cell lines transfected with DNA plasmids for research use only
To be received from Armour Therapeutics as per Material Transfer Agreement, March 2012

These cell lines are all transfectants of parental MDA MB 231 LucD3H2LN (also known as MDA-MB-D3H2-231LN) breast cancer cells, already listed on the Chambers BIO-LRCC-0022 permit.

MDA-MB-D3H2-231LN stably transfected with pATI-eGFP
MDA-MB-D3H2-231LN breast cancer cell line, stably transfected with **Armour's** DNA plasmid for expression of eGFP driven by a eukaryotic CMV Immediate Early (IE) promoter. eGFP expression is driven downstream of an Encephalomyocarditis Virus (EMCV) IRES element. Plasmid contains a Kan/Neo Resistance element.

This is a control transfectant, containing only Green Fluorescent Protein (eGFP), and we anticipate that it will be similar in properties to the parental MDA MB 231 LucD3H2LN cells.

MDA-MB-D3H2-231LN stably transfected with pATI-H2/eGFP
MDA-MB-D3H2-231LN breast cancer cell line, stably transfected with **Armour's** DNA plasmid for bi-cistronic expression of human H2 relaxin and eGFP driven by a eukaryotic CMV Immediate Early (IE) promoter. eGFP expression is driven downstream of an Encephalomyocarditis Virus (EMCV) IRES element. Plasmid contains a Kan/Neo Resistance element.

These cells are MDA MB 231 LucD3H2LN cells, transfected both with eGFP and the human H2 relaxin gene. We anticipate that these cells will be more malignant than the parental or control cells, based on the Silvertown 2007 reference, listed below.

MDA-MB-D3H2-231LN stably transfected with pATI-001/eGFP
MDA-MB-D3H2-231LN breast cancer cell line, stably transfected with **Armour's** DNA plasmid for bi-cistronic expression of AT-001, a human H2 relaxin analog, and eGFP driven by a eukaryotic CMV Immediate Early (IE) promoter. eGFP expression is driven downstream of an Encephalomyocarditis Virus (EMCV) IRES element. Plasmid contains a Kan/Neo Resistance element.

These cells are MDA MB 231 LucD3H2LN cells, transfected both with eGFP and an analog of the human H2 relaxin gene (AT-001). We anticipate that these cells will be less malignant than the parental or control cells, based on the Silvertown 2007 reference, listed below.

Reference
Silvertown JD, Symes J, Neschadim A, Nonaka T, Kao JC, Summerlee AJ, Medin JA. (2007). Analog of H2 relaxin exhibits antagonistic potential and suppresses prostate tumor growth. FASEB J, 21(3): 754-765.

RESEARCH SUMMARY:
The purpose of this project is to determine the role of the relaxin gene and a variant of this gene, on tumor cell malignancy. This project will test the hypothesis that the relaxin gene will enhance the malignancy of these cells, and that the relaxin variant will inhibit malignancy. This basic information will be important for future development of the relaxin variant as a new therapeutic approach for breast cancer.

PLEASE EXPLAIN HOW THE AGENTS ARE USED, STORED AND DISPOSED OF:
These three cell lines will be stored, used and disposed of as previously described for the parental MDA MB 231 LucD3H2LN cells, in BIO LRCP 0022, as follows:

***Storage**: Cell lines will be stored frozen at -80 and/or -150C.*

***Usage**: These cell lines will be maintained under routine tissue culture conditions. They will be grown in CO2 incubators, passaged and handled in a laminar flow biosafety cabinet. They will be used in vitro for proliferation and migration assays. They will be used in vivo by mammary fat pad injection to form primary tumors, under approved UWO animal protocol #2009-072. Tumor growth will be measured, and at endpoint primary tumors will be fixed in formalin for histological analyses.*

***Disposal**: Cell stocks will be bleached to kill the cells prior to disposal via the sewer. Plasticware that has been in contact with cells are placed in yellow biohazard bags inside cardboard boxes, for disposal by Stericycle (licensed waste carrier).*

The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards 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.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Ann F. Chambers and Alan Tuck (co PI)**
DEPARTMENT:	**Cancer Research Labs**
ADDRESS:	**LRCP Rm A4-903, 790 Commissioners Rd. E., London, ON**
PHONE NUMBER:	**519 685 8652**
EMERGENCY PHONE NUMBER(S):	**519 657 7166**
EMAIL:	**ann.chambers@lhsc.on.ca, atuck@uwo.ca**

Location of experimental work to be carried out :

Building :	**London Regional Cancer Program (LRCP)**	Room(s):	**A4-903, A4-925, A4-822**
Building :	**Victoria Research Labs/Tower**	Room(s):	**Animal Vivarium**
Building :		Room(s):	

***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 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **LRCP Small Grant. Lawson award. Lloyd Carr-Harris Foundation.**

GRANT TITLE(S): **Molecular basis of early breast cancer progression. Molecular basis of tumor dormancy. The role of the protein osteopontin (OPN) in cancer progression and predicting response to therapy.**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Pieter Anborgh, PhD	**pieter.anborgh@lhsc.on.ca**	**Jan 10, 2012**
Allen Clifford	**acliffo4@uwo.ca**	**Jan 10, 2012**
David Dales	**dwdales@uwo.ca**	**Dec 21, 2011**
Nicole Hague	**nhague07@gmail.com**	**Nov 23, 2011**

Connor MacMillian	cmacmil8@uwo.ca	Jan 10, 2012
Carl Postenka	cpostenk@uwo.ca	Jan 10, 2012
Caroline Trieman	caroline.trieman@schulich.uwo.ca	Sept 18, 2009

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

Biological Agents: human and murine cancer cell lines (list attached), Lentiviral Vector System from OPEN BioSystems, Bacteria

Usage: Cell lines are maintained under routine cell culture techniques.
(Grown in CO2 incubators, passaged and handled in a laminar flow BSC.
Cells are used in-vitro for 2D and 3D assays (ie proliferation, migration assay).

Cells for in-vivo mouse studies may be injected (tail vein, mammary fat pad, intercardiac) for observation of tumor development and metastisis. Mice will be handled and kept at the VRL vivarium which is a Level II animal-handling facility.

The Lentiviral Vector System will be used to transfect a shRNA library into cancer cells. The lentivirus contains a deletion in the LTR region which prevents transcription of the virus in transfected cell lines. The lentivirus is made by transfecting three different plasmids into cells that will make lentivirus particles. The cells typically used are called HEK293 cells. After 24-72 hrs post-transfection, media is collected which contains viral particles released by the HEK293 cells. This media, usually 2-3 mL is immediately transferred to another well which contains the cells that will be infected with virus. After 4 days of infection, this media is removed and bleached. No vacuum aspiration is used, only a disposable 10mL pipette is used and is bleached as well.
All viral transfection work will be performed only in room LRCP A4-822 which is designated as Level 2+ for viral work.

Bacterial strains, containing recombinant protein expression plasmid or vector DNA, are used in the mass production of protein or in the amplification of plasmid DNA.

Storage: Frozen at -20,-80 and -150C

Disposal: All items used in viral work are bleached prior to disposal.
Liquids are either autoclaved or bleached prior to being deposited into the sewer.
Plasticware that has been in contact with biological agent(s) are placed in yellow biohazard bags inside cardboard boxes. Disposable glassware is placed in yellow biohazard plastic pails. These biohazard boxes and pails are then taken away by Stericycle (licensed waste carrier) who then autoclaves the items prior to disposal in landfill.

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

Project 1: Molecular basis of early breast cancer progression. Breast cancer goes through stages of progression, from early-stage lesions, called atypical ductal hyperplasia (ADH) and ductal carcinoma in situ (DCIS), which can be easily treated, to invasive mammary carcinoma (IMC), which is much harder to treat successfully. We are using a series of established cell lines (the 21T series) from one breast cancer patient, which represent these three stages. We have identified a set of genes that are associated with these three stages, and we are testing if some of them can functionally contribute to breast cancer progression to IMC. To do this, we are over-expressing these genes in the 21T series of cells, or down-regulating them, as appropriate. We then will test to see if the behavior of the cells is changed, using both in vitro assays in tissue culture, as well as in vivo for tumor forming ability in mice. This work will help us to understand which early breast tumors are likely to progress to IMC, and may lead to treatment strategies to prevent this transition.

Representative reference: Souter LH, Andrews JD, Zhang G, Cook AC, Postenka CO, Al-Katib W, Leong HS, Rodenhiser DI, Chambers AF, Tuck AB. 2010. Human 21T breast epithelial cell lines mimic breast cancer progression in vivo and in vitro and show stage-specific gene expression patterns. Laboratory Investigation 90:1247-58.

Project 2: Molecular basis of tumor dormancy. Some breast cancers can recur long after a patient is thought to be cured of her cancer, because cancer cells have been dormant in the body. We have developed an in vitro cell culture test that can model dormant vs. active tumor growth. We are using this test to identify genes that contribute to cancer cell dormancy, by up- or down-regulating genes in mouse or human breast cancer cells. We have shown that cells that are dormant do not respond well to cancer chemotherapy, and are now asking if the same holds true for radiation therapy. This work will help us to understand how cancer cells may remain dormant in the body, what may cause them to start growing after a period of dormancy, and how they may be treated.

Representative reference: Barkan D, Kleinman H, Simmons JL, Asmussen H, Kamaraju AK, Hoenorhoff MJ, Liu Z-Y, Costes SV, Cho EH, Lockett S, Khanna C, Chambers AF, Green JE. 2008. Inhibition of metastatic outgrowth from single dormant tumor cells by targeting the cytoskeleton. Cancer Research 68: 6241-6250.

Project 3: The role of the protein osteopontin (OPN) in cancer progression and predicting response to therapy. We have shown that the protein OPN is associated with cancer progression, and functionally acts to make cells more aggressive. We are able to measure OPN in cancer patients' blood and tumors, and high levels are linked to poor patient outcome. Recently, we have reported that over-expression of OPN in breast cancer cells may predict better response to new targeted therapies. We are extending this work to a series of lung cancer cell lines, to learn if high levels of OPN in the cells predicts response to new targeted drugs that are being used in lung cancer therapy. This work may help to identify which patients will benefit from these new drugs, and which patients are unlikely to respond.

Representative reference: Mutrie JC, Tuck AB, Chambers AF. 2011. Osteopontin increases breast cancer cell sensitivity to specific signalling pathway inhibitors in preclinical models. Cancer Biology and Therapy 12:680-90.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other 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:
risk of spillage in which case the area will be cleaned using bleach.
Possible aerosol generation in which case work is performed in BSC.

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	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
DH5alpha	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1L**	**New England Biolabs**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
BL21	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1L**	**Pharmacia**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
JM109	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1L**	**Pharmacia**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Lentivirus	☒ Yes ☐ No	☒ Yes ☐ No	☒ Yes ☐ No	**0.025L**	**Open Biosystems**	☐ 1 ☐ 2 ☒ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: **Work with Level 2+ agents is conducted only in Rm A4-822**

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	☐ Yes ☒ No		Not applicable
Rodent	☐ Yes ☒ No		
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**see attached sheet**	**see attached sheet**	**attached sheet**
Rodent	☒ Yes ☐ No	**see attached sheet**	**see attached sheet**	**attached sheet**
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ 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 ☐ 2+ ☐ 3

Additional Comments: _____

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 Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	**cancer patient whole blood**	☐ Yes ☒ Unknown		☒ 1 ☐ 2 ☐ 2+ ☐ 3
Human Blood (fraction) or other Body Fluid	**cancer patient plasma**	☐ Yes ☒ Unknown		☒ 1 ☐ 2 ☐ 2+ ☐ 3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐ 1 ☐ 2 ☐ 2+ ☐ 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: **UWO Research Ethics Review # 15925**

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
DH5alpha	**see attached list**	**see attached list**	**see attached list**	**no**	**no**	**amplification of plasmid DNA**

** Please attach a Material Safety Data Sheet or equivalent if available.*
*** Please attach a plasmid map.*
****No Material Safety Data Sheet is required for the following strains of E. coli:*
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells 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 from transduction
Lentivirus	**pGIPZmir library**	**Open Biosystems**	**complete genome library**	**decrease in gene expression**

** Please attach a Material Safety Data Sheet or equivalent.*

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☒ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☐ NO ☒ YES, specify **HIV Enhancer sequence**
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: **Viral work (Level 2+) is only conducted in Rm A4-822. No viral work done in Rms A4-903/925 which are classified as Level 2.**

6.0 Human Gene Therapy Trials

6.1 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 7.0

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

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **mouse**

7.3 AUS protocol # **2009-072, 2009-080, 2010-019**

7.4 List the location(s) for the animal experimentation and housing. **Victoria Research Labs Vivarium**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☒ YES, specify: **stable transfected cancer cell lines injected into mice**

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: injection into mammary fat pad for tumour formation with unexpected clearance of cells from fat pad

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

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

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree…)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 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 biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name _____ ***Date:*** ____

Ann F. Chambers Ann F. Chambers Jan 24, 2012
Alan B. Tuck Alan B. Tuck Jan 24/2012

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☐ 1 ☐ 2 ☒ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **Rms A4-903/925 was Dec. 2010, Rm A4-822 was Dec ~~2009~~** Dec. 2010
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **R-06-000599 & BIO-LRCC-0022**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Splash to eyes: Immediately flush eyes with running water for 15 minutes using eyewash and forcibly hold eye(s) open to ensure effective wash behind the eyelids. Needle stick: Wash affected area thoroughly using antimicrobial soap for 5 minutes. Exposed work area will be cleaned with bleach. Incident to be reported to OHS/LHSC and for treatment if necessary.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Ann F. Chambers ***Date:*** Jan 24, 2012

15.4 Additional Comments: Alan B. Tuck Jan 24, 2012

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: March 1 2012

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Mar 8 2012

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Gail Ryder
Date: January 25, 2012

Approval Number: B10-LRCC-0022 Expiry Date (3 years from Approval): March 16, 2015
B10-LRCC-0009

Special Conditions of Approval:

ATTACHMENTS

Section 1.2

MSDS

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie

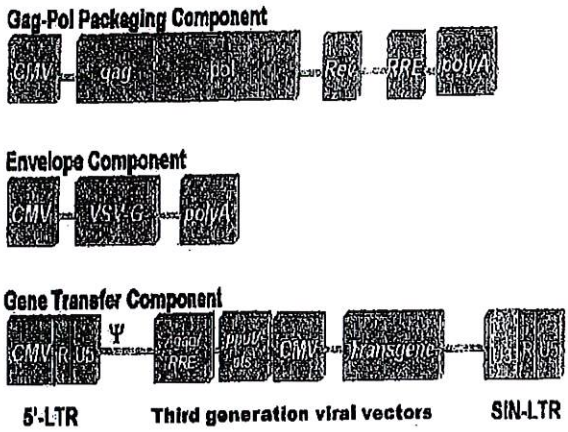
Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

OPEN BIOSYSTEMS

Safety and handling of the shRNAmir Lentiviral Vector System

Typical 'third generation' lentiviral vectors provide a three part packaging system that does have the unwanted potential for production of replication competent retrovirus. These lentiviral vector stocks have been shown to recombine during reverse transcription in the targeted cells. This will re-join the viral protein-coding sequences of the packaging construct and *cis*-acting sequences of the vector generating *env*-minus recombinants (LTR-*gag-pol*-LTR). Mobilization of recombinant lentiviral genomes was also demonstrated but was dependent on pseudotyping of the vector core with an exogenous envelope protein. 5′ sequence analysis has demonstrated that recombinants consist of U3, R, U5, and the Ψ packaging signal joined with an open *gag* coding region. Analysis of the 3 end has mapped the point of vector recombination to the poly (A) tract of the packaging construct's mRNA.[4]

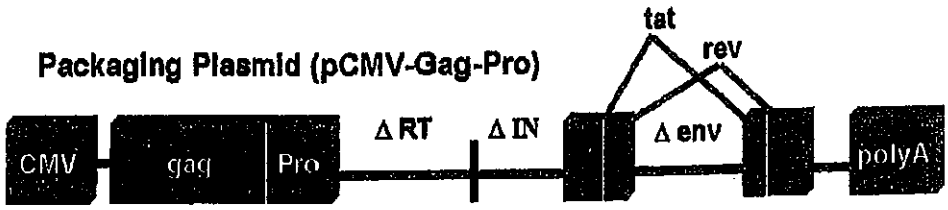


The Trans-Lentiviral™ packaging system is designed with the latest and best safety technologies of any expression system. The trans-lentiviral packaging vectors, a new class of HIV-based vectors, enhance the third-generation system by splitting the *gag-pol* component of the packaging construct into two parts. The first expresses *Gag/Gag-Pro*. The second expresses *Pol* (RT and IN) fused with *Vpr*. Unlike other lentiviral vectors, the trans-lentiviral vectors do not form recombinants capable of DNA mobilization. These results indicate the Trans-vector™ design prevents the generation of *env*-minus recombinant lentivirus containing a functional *gag-pol* structure (LTR-*gag-pol*-LTR), which is absolutely required for retroviral DNA mobilization.

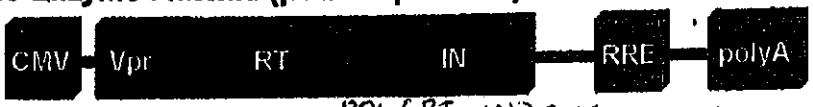
OPEN BIOSYSTEMS

Trans-Lentiviral™ packaging system components

Packaging Plasmid (pCMV-Gag-Pro)



Trans-Enzyme Plasmid (pCMV-Vpr-RT-IN)



POL (RT-IN) FUSED to Vpr

Env-Coding Plasmid (pCMV-VSV-G)

Gene Transfer Vector

self inactivating U3 deleted and this inhibits RNA production in transduced cells

The Expression Arrest™ shRNAmir lentiviral vectors are **self inactivating (sin)** expression vectors. The viral vectors contain a specifically designed long terminal repeat (LTR) derived from the native virus. These LTR's differs from the native by several point mutations and a deletion, enhancing transcriptional activation and decreasing transcriptional suppression in embryonic stem and embryonal carcinoma cells.

Self inactivating vectors are constructed by deleting the enhancer and/or the promoter in the U3 region of the 3' LTR. During reverse transcription, a circular intermediate is formed that transfers the deletion to the 5' LTR of the pro-viral DNA. The deletion abolishes any transcriptional activity driven by the LTR so that no full-length vector RNA is produced in transduced cells. Following a single round of replication, the changes are copied into both 5' and 3' LTRs resulting in inactive provirus. However, any promoter internal to the LTRs in such vectors will still be active. This strategy has been employed to eliminate effects of the enhancers and promoters in the viral LTRs on transcription from internally placed genes.

The Expression Arrest™ shRNAmir lentiviral vectors are infectious **only** when packaged in a cell line with appropriate tropism but is not replication competent. Virus produced by both transient and stable transfections can infect target cells and transmit target genes; however, it cannot replicate within target cells because the viral structural genes are absent.

OPEN BIOSYSTEMS

Viral packaging

To effectively package lentivirus, the viral gag, pol and env genes- necessary for particle formation and replication- are co-transfected into the packaging cell line. The separate introduction of the structural genes minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation. Viral expression vectors provide the packaging signal, transcription and processing elements, and a target gene or shRNA. Simultaneous transfection of the gene transfer vector and viral packaging plasmids into a packaging cell line produces high-titer, replication-incompetent virus.

The protocols for viral cell packaging require the producing, handling and storing of infectious lentivirus. An understanding of safe laboratory practices and potential viral hazards is necessary. Appropriate NIH, regional, and institutional guidelines apply, as well as specific guidelines for other countries. In the United States, NIH guidelines require that viral production and transduction be performed in a Biosafety Level 2 (BL2) facility for more information about BL2 guidelines the section below is provided (http://bmbl.od.nih.gov/contents.htm)

Excerpt from the NIH Recommendations on Biosafety in Biomedical and Microbiological Laboratories

Biosafety Level 2 (BSL-2) is similar to Biosafety Level 1 (BSL-1) and is suitable for work involving agents of moderate potential hazard to personnel and the environment. It differs from BSL-1 in that (1) laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists; (2) access to the laboratory is limited when work is being conducted; (3) extreme precautions are taken with contaminated sharp items; and (4) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following standard and special practices, safety equipment, and facilities apply to agents assigned to Biosafety Level 2:

A. Standard Microbiological Practices

1. Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments are in progress.
2. Persons wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
3. Eating, drinking, smoking, handling contact lenses, and applying cosmetics are not permitted in the work areas. Food is stored outside the work area in cabinets or refrigerators designated for this purpose only.

4. Mouth pipetting is prohibited; mechanical pipetting devices are used.
5. Policies for the safe handling of sharps are instituted.
6. All procedures are performed carefully to minimize the creation of splashes or aerosols.
7. Work surfaces are decontaminated on completion of work or at the end of the day and after any spill or splash of viable material with disinfectants that are effective against the agents of concern.
8. All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method such as autoclaving. Materials to be decontaminated outside of the immediate laboratory are placed in a durable, leakproof container and closed for transport from the laboratory. Materials to be decontaminated off-site from the facility are packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.
9. An insect and rodent control program is in effect (see Appendix G).

B. Special Practices

1. Access to the laboratory is limited or restricted by the laboratory director when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may have serious consequences, are not allowed in the laboratory or animal rooms. For example, persons who are immunocompromised or immunosuppressed may be at increased risk of acquiring infections. The laboratory director has the final responsibility for assessing each circumstance and determining who may enter or work in the laboratory or animal room.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements (e.g., immunization) may enter the laboratory.
3. A biohazard sign must be posted on the entrance to the laboratory when etiologic agents are in use. Appropriate information to be posted includes the agent(s) in use, the biosafety level, the required immunizations, the

investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.

4. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
5. When appropriate, considering the agent(s) handled baseline serum samples for laboratory and other at-risk personnel are collected and stored. Additional serum specimens may be collected periodically, depending on the agents handled or the function of the facility.
6. Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of special hazards and are required to read and follow instructions on practices and procedures.
7. The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the work involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
8. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
    a. Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as parenteral injection, phlebotomy, or aspiration of fluids from laboratory animals and diaphragm bottles. Plastic ware should be substituted for glassware whenever possible.
    b. Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be

carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination, preferably by autoclaving.

c. Syringes which re-sheathe the needle, needle less systems, and other safety devices are used when appropriate.

d. Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal, according to any local, state, or federal regulations.

9. Cultures, tissues, specimens of body fluids, or potentially infectious wastes are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
10. Laboratory equipment and work surfaces should be decontaminated with an effective disinfectant on a routine basis, after work with infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility.
11. Spills and accidents that result in overt exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
12. Animals not involved in the work being performed are not permitted in the lab.

C. Safety Equipment (Primary Barriers)

1. Properly maintained biological safety cabinets, preferably Class II, or other appropriate personal protective equipment or physical containment devices are used whenever:

a. Procedures with a potential for creating infectious aerosols or splashes are conducted. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals intranasally, and harvesting infected tissues from animals or embryonate eggs.

b. High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory if sealed rotor heads or centrifuge safety cups are used, and if these rotors or safety cups are opened only in a biological safety cabinet.

2. Face protection (goggles, mask, face shield or other splatter guard) is used for anticipated splashes or sprays of infectious or other hazardous materials to the face when the microorganisms must be manipulated outside the BSC.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. This protective clothing is removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing is either disposed of in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves are worn when hands may contact potentially infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate. Gloves are disposed of when overtly contaminated, and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Disposable gloves are not washed, reused, or used for touching "clean" surfaces (keyboards, telephones, etc.), and they should not be worn outside the lab. Alternatives to powdered latex gloves should be available. Hands are washed following removal of gloves.

OPEN BIOSYSTEMS

D. Laboratory Facilities (Secondary Barriers)

1. Provide lockable doors for facilities that house restricted agents (as defined in 42 CFR 72.6).
2. Consider locating new laboratories away from public areas.
3. Each laboratory contains a sink for hand washing. Foot, knee, or automatically operated sinks are recommended.
4. The laboratory is designed so that it can be easily cleaned. Carpets and rugs in laboratories are inappropriate.
5. Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surfaces and equipment.
6. Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning. Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
7. Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment. Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
8. An eyewash station is readily available.
9. Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
10. There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory. If the laboratory has windows that open to the exterior, they are fitted with fly screens.

OPEN BIOSYSTEMS

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 trans-lentiviral™ packaging system.

Note: Viral supernatants produced using lentiviral packaging plasmids, depending on your shRNA insert, may contain potentially hazardous recombinant virus. All users must exercise caution in the production, use and storage of recombinant virus, especially those with amphotropic or dualtropic host ranges.

OPEN BIOSYSTEMS

References

1. Chen W, Wu X, Levasseur DN, Liu H, Lai L, Kappes JC, Townes TM.
Lentiviral vector transduction of hematopoietic stem cells that mediate long-term reconstitution of lethally irradiated mice.
Stem Cells. 2000;18(5):352-9.
PMID: 11007919

2. Coffin, JM, Hughes SH and Varmus H. Eds.
Retroviruses
1997 Cold Spring Harbor Laboratory Press.

3. Kappes JC, Wu X, Wakefield JK.
Production of trans-lentiviral vector with predictable safety.
Methods Mol Med. 2003;76:449-65.
PMID: 12526179

4. Kappes JC, Wu X.
Safety considerations in vector development.
Somat Cell Mol Genet. 2001 Nov;26(1-6):147-58.
PMID: 12465466

5. Wu X, Wakefield JK, Liu H, Kappes JC.
Analysis of lenti- and trans-lentiviral vector genetic recombination.
Dev Biol (Basel). 2001;106:237-48; discussion 249, 253-63.
PMID: 11761237

6. Wu X, Wakefield JK, Liu H, Xiao H, Kralovics R, Prchal JT, Kappes JC.
Development of a novel trans-lentiviral vector that affords predictable safety.
Mol Ther. 2000 Jul;2(1):47-55.
PMID: 10899827

Expression Arrest™ GIPZ lentiviral shRNAmir-Glycerol Stocks

APPLICABLE CATALOG NUMBERS

Catalog Numbers	Description
RHS4430	Human GIPZ lentiviral shRNAmir individual clone
RMM4431	Mouse GIPZ lentiviral shRNAmir individual clone
RHS4346	Non-silencing-GIPZ lentiviral shRNAmir control-Glycerol stock
RHS4349	pGIPZ lentiviral empty vector - Glycerol stock
RHS4371	GAPDH-GIPZ lentiviral shRNAmir positive control-Glycerol stock
RHS4480	EG5-GIPZ lentiviral shRNAmir positive control-Glycerol stock
RHS4477	Human GIPZ lentiviral shRNAmir library subscription
RMM4501	Mouse GIPZ lentiviral shRNAmir library subscription

PRODUCT DESCRIPTION
The GIPZ lentiviral shRNAmir library was developed by Open Biosystems in collaboration with Dr. Greg Hannon (CSHL) and Dr. Steve Elledge (Harvard). This library combines the design advantages of microRNA-adapted shRNA (shRNAmir) with the pGIPZ lentiviral vector to create a powerful RNAi trigger capable of producing RNAi in most cell types including primary and non-dividing cells.

QUALITY CONTROL
Open Biosystems checks all cultures for growth prior to shipment.

SHIPPING AND STORAGE
Individual constructs are shipped as bacterial cultures of *E. coli* (Prime Plus) in LB-Lennox (low salt) broth with 8% glycerol, 100µg/ml carbenicillin and 25µg/ml zeocin. Individual constructs are shipped on wet ice. Collections are shipped in 96 well plate format on dry ice. Individual constructs and collections should be stored at -80°C.

TO ALLOW ANY CO_2 THAT MAY HAVE DISSOLVED INTO THE MEDIA FROM THE DRY ICE IN SHIPPING TO DISSIPATE, PLEASE STORE CONSTRUCTS AT –80°C FOR AT LEAST 48 HOURS BEFORE THAWING.

Important Safety Note:
Follow NIH guidelines regarding lentiviral production and transduction; follow Biosafety Level 2 (BL2) or BL2+ laboratory criteria.
NIH Agent Summary Statement: http://bmbl.od.nih.gov/viral2.htm#retro
NIH Biosafety Level 2 Description: http://bmbl.od.nih.gov/sect3bsl2.htm
NIH/RAC "Guidance on Biosafety Considerations for Research with Lentiviral Vectors":
http://www4.od.nih.gov/oba/RAC/Guidance/LentiVirus_Containment/pdf/Lenti_Containment_Guidance.pdf

Please note that GIPZ vectors are not compatible with third generation packaging systems such as ViraPower from Invitrogen. We recommend the TransLentiviral Packaging system for use with our vectors.

PRODUCT INFORMATION
The product manual for the Expression Arrest pGIPZ lentiviral shRNAmir collection is available for download using the following link:
https://www.openbiosystems.com/collateral/rnai/pi/pGIPZ-manual.pdf

ThermoFisher SCIENTIFIC

Material Safety Data Sheet

Creation Date 18-Sep-2009 **Revision Date** 24-May-2010 **Revision Number** 2

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Viral Particles - Including GIPZ, Lenti-ORF, and shMIMIC

Cat No. VGHXXXX, VGMXXXX, VGRXXXX, RHS4348, RHS4372, RHS 4351, HMRXXXX, VSHXXXX (Excluding Arrayed Libraries), OHSXXXX

Synonyms No information available.

Recommended Use For research use only

Company
Thermo Fisher Scientific
Open Biosystem Products
601 Genone Way # 2100
Huntsville, AL 35806 United States
Tel: (303) 604-9499
Fax:(303) 604-9680

Emergency Telephone Number
Chemtrec US: (800) 424-9300
Chemtrec EU: (202) 483-7616

2. HAZARDS IDENTIFICATION

WARNING!

Emergency Overview

Potential Biohazard. Handle in accordance with good industrial hygiene and safety practice. May cause eye, skin, and respiratory tract irritation . Shipped on dry ice.

Appearance Yellow **Physical State** Liquid **odor** No information available

Target Organs None known.

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	May cause irritation
Skin	May cause irritation
Inhalation	May cause irritation of respiratory tract
Ingestion	Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea

Chronic Effects None known.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
DMEM	NA	1 - 99
Viral Particles	NA	1 - 99

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire..

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
None known.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA	Health 1	Flammability 0	Instability 0	Physical hazards N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions	Use personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing.
Environmental Precautions	Should not be released into the environment.
Methods for Containment and Clean Up	Soak up with inert absorbent material. Keep in suitable and closed containers for disposal.

7. HANDLING AND STORAGE

Handling	Handle in accordance with good industrial hygiene and safety practice. Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation. This material should be handled at the biosafety level 2 (BSL2) as required by OSHA Bloodborne Pathogen Rule (29 CFR 1910.1030.7).
Storage	Keep container tightly closed. Keep at -80°C.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures	Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.
Exposure Guidelines	This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

NIOSH IDLH: Immediately Dangerous to Life or Health

Personal Protective Equipment

Eye/face Protection	Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166
Skin and body protection	Wear appropriate protective gloves and clothing to prevent skin exposure
Respiratory Protection	Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Yellow
odor	No information available
Odor Threshold	No information available.
pH	Not applicable
Vapor Pressure	No information available.
Vapor Density	No information available.

9. PHYSICAL AND CHEMICAL PROPERTIES

Viscosity	No information available.
Boiling Point/Range	Not applicable
Melting Point/Range	No information available.
Decomposition temperature	No information available.
Flash Point	Not applicable
Evaporation Rate	No information available.
Specific Gravity	No information available.
Solubility	No information available.
log Pow	No data available

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Conditions to Avoid	Excess heat.
Incompatible Materials	None known
Hazardous Decomposition Products	None known
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information No acute toxicity information is available for this product

Component Information

Irritation No information available.

Toxicologically Synergistic Products No information available.

Chronic Toxicity

Carcinogenicity There are no known carcinogenic chemicals in this product

Sensitization No information available.

Mutagenic Effects No information available.

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity	No information available.
Other Adverse Effects	The toxicological properties have not been fully investigated..
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Do not empty into drains.

Persistence and Degradability	No information available
Bioaccumulation/ Accumulation	No information available
Mobility	No information available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods	Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

TDG

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IATA

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IMDG/IMO

14. TRANSPORT INFORMATION

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

15. REGULATORY INFORMATION

International Inventories

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
Not Applicable

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know
Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
D3 Biohazardous infectious materials

16. OTHER INFORMATION

Prepared By	Regulatory Affairs Thermo Fisher Scientific Tel: (412) 490-8929
Creation Date	18-Sep-2009
Print Date	24-May-2010
Revision Summary	"****", and red text indicates revision

Disclaimer

The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

Section 2.3

Cell Lines Grown in Culture

And

MSDS

Biological Agents Registry Form
Jan. 2012

2.3 Established cells that will be grown in culture

Specific Cell Line	Containment Level	Supplier/Source of cell line	MSDS	Link to publication
Human				
Mammary Cancer Cell lines				
21 T series (PT, NT, MT-1) mammary epithelial -transfected parental 21T cells:	1	Dana Farber Res. Inst.	N/A	PMID:1977518
21 NT/shS100A2	1	Chambers' laboratory	N/A	
21 NT/Empty vector (EV)	1	Chambers' laboratory	N/A	
21 PT/shS100A2	1	Chambers' laboratory	N/A	
21 PT/Empty vector (EV)	1	Chambers' laboratory	N/A	
21 NT/EV (cDNA3.1myc/his)	1	Chambers' laboratory	N/A	
21 PT/EV (cDNA3.1myc/his)	1	Chambers' laboratory	N/A	
21 NT/pZsGreen1 (ZsG) vector	1	Chambers' laboratory	N/A	
21 NT/TBX3 iso1/ZsG	1	Chambers' laboratory	N/A	
21 NT/TBX3 iso2/ZsG	1	Chambers' laboratory	N/A	
21 PT/VANGL1	1	Chambers' laboratory	N/A	
21 NT/WNT5A	1	Chambers' laboratory	N/A	
21 PT/WNT5A	1	Chambers' laboratory	N/A	
21 NT/shLuciferase	1	Chambers' laboratory	N/A	
21 PT/shLuciferase	1	Chambers' laboratory	N/A	
21 MT-1/shLuciferase	1	Chambers' laboratory	N/A	
21 NT/EV/shLuciferase	1	Chambers' laboratory	N/A	
21 PT/EV/shLuciferase	1	Chambers' laboratory	N/A	
21 PT/VANGL1/shLuciferase	1	Chambers' laboratory	N/A	
21 PT/shWNT5A	1	Chambers' laboratory	N/A	
21 PT/EV/shWNT5A	1	Chambers' laboratory	N/A	
21 PT/VANGL1/shWNT5A	1	Chambers' laboratory	N/A	
21 MT-1/shWNT5A	1	Chambers' laboratory	N/A	
21 NT/shVANGL1	1	Chambers' laboratory	N/A	
21 NT/EV/shVANGL1	1	Chambers' laboratory	N/A	
21 NT/WNT5A/shVANGL1	1	Chambers' laboratory	N/A	
21 MT-1/shVANGL1	1	Chambers' laboratory	N/A	
MDA MB 175	1	A.T.C.C.	see attached page	
MDA MB 231	1	A.T.C.C.	see attached page	
MDA MB 231 Luc D3H2LN	1	Caliper Life	N/A	
MDA MB 435	1	A.T.C.C.	see attached page	
MDA MB 468	1	A.T.C.C.	see attached page	
MDA MB 468 CON	1	Chambers' laboratory	N/A	PMID:16816376
MDA MB 468 OPN	1	Chambers' laboratory	N/A	PMID:16816376

Embryonic Kidney Cell line				
HEK-293	2	A.T.C.C.	see attached page	
Colon Cancer Cell line				
HT-29	1	A.T.C.C.	see attached page	
Lung Cancer Cell lines				
NCI-H1648	1	A.T.C.C.	see attached page	
-transfected H1648 cells:				
H1648/pcDNA3.1	1	Chambers' laboratory	N/A	
H1648/pcDNA3hOPNa	1	Chambers' laboratory	N/A	
H1648/pSUPER-NM636	1	Chambers' laboratory	N/A	
H1648/pSUPER-scrambled	1	Chambers' laboratory	N/A	
NCI-H1650	1	A.T.C.C.	see attached page	
-transfected H1650 cells:				
H1650/pcDNA3.1	1	Chambers' laboratory	N/A	
H1650/pcDNA3hOPNa	1	Chambers' laboratory	N/A	
NCI-H1792	1	A.T.C.C.	see attached page	
-transfected H1792 cells:				
H1792/pcDNA3.1	1	Chambers' laboratory	N/A	
H1792/pcDNA3hOPNa	1	Chambers' laboratory	N/A	
H1792/pSUPER-NM636	1	Chambers' laboratory	N/A	
H1792/pSUPER-scrambled	1	Chambers' laboratory	N/A	

Murine

Melamona Cancer Cell lines				
B16F1	1	A.T.C.C.	see attached page	
B16F10	1	A.T.C.C.	see attached page	
Mammary Cancer Cell lines				
D2A1	1	Fred Miller's lab	N/A	PMID:1586590
D2.0R	1	Fred Miller's lab	N/A	PMID:1586590
Sarcoma Cancer Cell line				
PAP2	1	Chambers' laboratory	N/A	PMID:4063960
Hybridoma Cell lines				
hybridoma mAb53	1	Chambers' laboratory	N/A	PMID:8083234
hybridoma mAb87-B	1	Chambers' laboratory	N/A	PMID:8083234

Additional Information for Established Cell Lines:

1) All cell lines are mycoplasma free and are routinely tested for mycoplasma.
2) Murine derived cell lines have been tested for pathogens at University of Missouri's Research Animal Diagnostic Laboratory (RADIL) by means of IMPACT PCR Profile.
   No pathogens were detected.
3) A recombinant retrovirus will be used to transduce a gene of interest into cell lines. During the initial stage of transduction the cells have to be treated as Level 2 containment. The transduced gene of interest will be integrated into the host genome and propagated as an endogenous gene. After several passages with a selection agent to establish a stable transduced cell line, no viable recombinant retrovirus will be present in the cell line and therefore the cell line can be handled as Level 1 containment.

Info on Cells

ATCC™

MATERIAL SAFETY DATA SHEET

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

MATERIAL SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Animal Cell Cultures at Biosafety Level 1 or 2
ATCC Catalog #: Various

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating:	Health: 0	Flammability: 0	Reactivity:0
NFPA Rating:	Health:0	Flammability:0	Reactivity:0

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

For Biosafety Level 1 Cell Cultures
Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.
This cell line is not known to cause disease in healthy adult humans. These cells have **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

See next page for Biosafety Level 2 cell cultures.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8. Wear laboratory coat, gloves and eye protection. Avoid all contact.

Methods for Cleaning Up

Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.
Equipment/Environment: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time before clean up (30 min).
Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:
Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use fume hood or other appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

missing pages

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

Cell Biology

ATCC® Number:	**HTB-25™**	Order this Item	Price:	**$429.00**

Designations:	MDA-MB-175-VII
Depositors:	R Cailleau
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	loosely adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Tissue:** duct **Disease:** ductal carcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
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.
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 7,8 D13S317: 11,12 D16S539: 11,13 D5S818: 11 D7S820: 11,12 THO1: 7 TPOX: 6,8 vWA: 15,16
Cytogenetic Analysis:	model number = 84; range = 82 to 89. The cell line is aneuploid female (XX/XXX/XXXX), with chromosome counts in the hypertetraploid to near-tetraploid range. All normal chromosomes are represented by at least one copy, with most chromosomes having three or four copies present per karyotype. Five marker chromosomes are found: del(1)p35), der(7)t(1;7)(q11;q32), unknown, t(2q;?), unknown isochromosome. Several are present in multiple copy. Alterations in the q arm of chromosome N1 are in accordance with the report by M.J. Siciliano, et al., Cancer Res. 39: 919, 1979.
Isoenzymes:	

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Cell Biology

ATCC® Number:	**HTB-26™**	Order this Item	Price:	**$279.00**

Designations:	MDA-MB-231
Depositors:	R Cailleau
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
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.
Applications:	transfection host
Receptors:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 12,13 D13S317: 13 D16S539: 12 D5S818: 12 D7S820: 8,9 THO1: 7,9.3 TPOX: 8,9 vWA: 15,18
Cytogenetic Analysis:	The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.
Isoenzymes:	

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ATCC® Number:	**HTB-129™**	Order this Item	Price:	**$279.00**

Designations:	MDA-MB-435S
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	spindle shaped 
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion
Cellular Products:	tubulin; actin
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:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18
Cytogenetic Analysis:	modal number = 56; range = 55 to 62 The cell line is aneuploid human female (XX), with most chromosome counts in the 55 to 60 range. Normal chromosomes N6, N11, and N22 were absent, while chromosomes N7, N13, N18 and N21 were single. Most of the remainder of normal chromosomes were usually paired, but chromosome N2 was triple. Nineteen marker chromosomes were identified, with most of them formed from structural alterations of the missing copies of the normal chromosomes. Six of these markers involve regions of chromosome N7, while three are recognized as derivatives of chromosome N6. Regions of a third copy of the normal and paired chromosomes N3, N15, N17, N20 are noted in markers M1, M2, M15, and M5, respectively.

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ATCC® Number:	**HTB-132™**	Order this Item	Price: **$279.00**

Designations:	MDA-MB-468
Depositors:	R Cailleau
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma
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:** 1977
Applications:	transfection host
Receptors:	epidermal growth factor (EGF) transforming growth factor alpha (TGF alpha)
Tumorigenic:	Yes
Antigen Expression:	Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 12 D16S539: 9 D5S818: 12 D7S820: 8 THO1: 7 TPOX: 8,9 vWA: 18
Cytogenetic Analysis:	modal number = 64; range = 60 to 67. The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.; A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes.; Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.

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Cell Biology

ATCC® Number:	**CRL-1573™**	Order this Item	Price:	**$279.00**

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney
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.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing transfection host viruscide testing
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Age:	fetus

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ATCC® Number:	**HTB-38™**	Order this Item	Price:	**$279.00**

Designations:	HT-29
Depositors:	J Fogh
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial 
Source:	**Organ:** colon **Disease:** colorectal adenocarcinoma
Cellular Products:	secretory component of IgA; carcinoembryonic antigen (CEA); transforming growth factor beta binding protein; mucin
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.
Restrictions:	The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.
Isolation:	**Isolation date:** 1964
Applications:	transfection host (Roche Transfection Reagents)
Receptors:	human adrenergic alpha2A [23560] urokinase receptor (u-PAR) vitamin D (moderate expression) urokinase receptor (u-PAR); vitamin D (moderate expression)
Tumorigenic:	Yes
Oncogene:	myc +; ras +; myb +; fos +; sis +; p53 +; abl -; ros -; src -
Antigen Expression:	Blood Type A; Rh+; HLA A1, A3, B12, B17, Cw5

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Cell Biology

ATCC® Number:	**CRL-5882™**	Order this Item	Price:	**$429.00**

Designations:	NCI-H1648 [H1648]
Depositors:	AF Gazdar, JD Minna
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	
Source:	**Organ:** lung **Tumor Stage:** stage 3A **Disease:** adenocarcinoma **Derived from metastatic site:** lymph node
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.
Restrictions:	The line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 12 D16S539: 11 D5S818: 11 D7S820: 10,11 THO1: 7,9.3 TPOX: 8,11 vWA: 14,17
Age:	39 years
Gender:	male
Ethnicity:	Black

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BioStandards

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Cell Biology

ATCC® Number:	**CRL-5883™**	Order this Item	Price:	**$429.00**

Designations:	NCI-H1650 [H-1650, H1650]
Depositors:	AF Gazdar, JD Minna
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** lung **Tumor Stage:** stage 3B **Disease:** adenocarcinoma; bronchoalveolar carcinoma **Derived from metastatic site:** pleural effusion
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.
Restrictions:	The line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.
Isolation:	**Isolation date:** May, 1987
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 11 D16S539: 11,12 D5S818: 11 D7S820: 8,9 THO1: 9.3 TPOX: 11 vWA: 18
GenoType:	EGFR (DelE746A750) [90471]
Age:	27 years

Related Links

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BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

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BioStandards

Biological Reference Material and Consensus Standards for the life science community

Cell Biology

ATCC® Number:	**CRL-5895™**	Order this Item	Price:	**$429.00**

Designations:	NCI-H1792 [H1792]
Depositors:	AF Gazdar, JD Minna
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** lung **Tumor Stage:** stage 4 **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion
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.
Restrictions:	The line is available with the following restrictions: 1. This cell line was deposited at the ATCC by Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 12 D16S539: 10,11 D5S818: 12 D7S820: 8,11 THO1: 7 TPOX: 11 vWA: 14,16
Age:	50 years
Gender:	male
Ethnicity:	Caucasian

Related Links ▸

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Cell Biology

ATCC® Number:	**CRL-6323™**	Order this Item	Price:	**$279.00**

Designations:	B16-F1
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	mixture of spindle-shaped and epithelial-like cells
Source:	**Organ:** skin **Strain:** C57BL/6J **Disease:** melanoma
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.
Applications:	transfection host (Roche Transfection Reagents)
Tumorigenic:	Yes
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. **Subcultivation Ratio:** A subcultivation ratio of 1:10 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** culture medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020

Related Links ▸

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Technical Support
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Cell Biology

ATCC® Number:	**CRL-6475™**	Order this Item	Price:	**$279.00**

Designations:	B16-F10
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus*
Morphology:	mixture of spindle-shaped and epithelial-like cells 
Source:	**Organ:** skin **Strain:** C57BL/6J **Disease:** melanoma
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.
Applications:	transfection host
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. 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 **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

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Product Information Sheet

BioProducts

Cell, microbial and molecular genomics products for the life sciences

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CELL LINES

Bioware Cell Line MDA-MB-231-luc-D3H2LN

Designation:	MDA-MB-231-luc-D3H2LN
Tissue	Human: adenocarcinoma; mammary gland; pleura effusion
Parental Line Source:	MD Anderson Cancer Center, the University of Texas
Co-Trasnfection Plasmids:	1) pGL3 control red (SV40-luc) (Promega/C. Contag, Stanford University) 2) pSV40/Zeo (Invitrogen)
Transfection Method:	Lipofectamine/Plus Reagent (Invitrogen)
Bioluminescence *In Vitro*:	Approximately 189-208 photons/second/cell, subject to imaging and culturing conditions
Passage:	A spontaneous lymph node metastasis from a D3H1 mammary fat pad tumor

The Features

Caliper Life Sciences Bioware Cell Line Models Offer the Ability to:

- Monitor early tumor development
- Monitor tumor growth and metastases *in vivo*
- Quantify tumor burden in the whole animal
- Follow responses to therapeutic treatments non-invasively in longitudinal studies using the same cohorts of mice.

Murine Pathogen Free

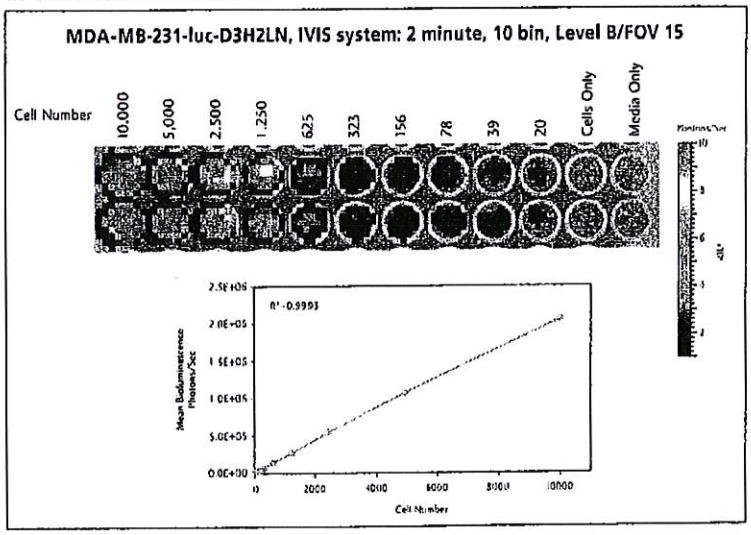
All Caliper Life Sciences cell lines are confirmed to be pathogen free by the IMPACT Profile I (PCR) at the University of Missouri Research Animal Diagnostic and Investigative Laboratory.

Model Description

MDA-MB-231-luc-D3H2LN is a luciferase expressing cell line that was derived from MDA-MB-231 human adenocarcinoma cells by stable transfection for the North American Firefly Luciferase gene expressed from the SV40 promoter. MDA-MB-231-luc-D3H2LN cells are derived from a spontaneous lymph node metastasis from a D3H1 mammary fat pad tumor. This cell line can be used *in vivo* to establish:

- Experimental Metastasis model (intravenous) and Intracardiac
- Orthotopic mammary fat pad model with metastasis

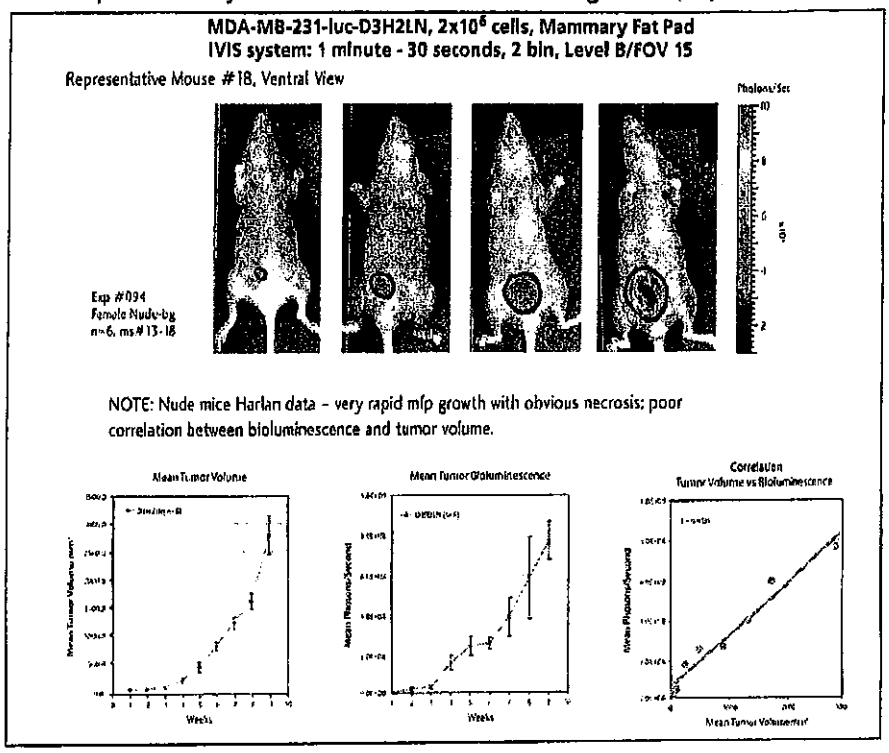
In Vitro Bioluminescence



Protocols:
MDA-MB-231-luc-D3H2LN cells in 100 µL media were seeded into a 96 well plate by 1:2 serial dilution from 10,000 cells (well#1) to 20 cells (well#10). The plate was imaged using the IVIS system (2 min, 10 bin, level B/FOV 15) approximately 2-3 minutes after addition of 100 µL 2X luciferin. Wells #11 and #12 served as negative controls.

Conclusions:
Approximately 20 cells were detectable in vitro in this experiment. A strong correlation between cell number and bioluminescence (R2 = 0.999) was also demonstrated.

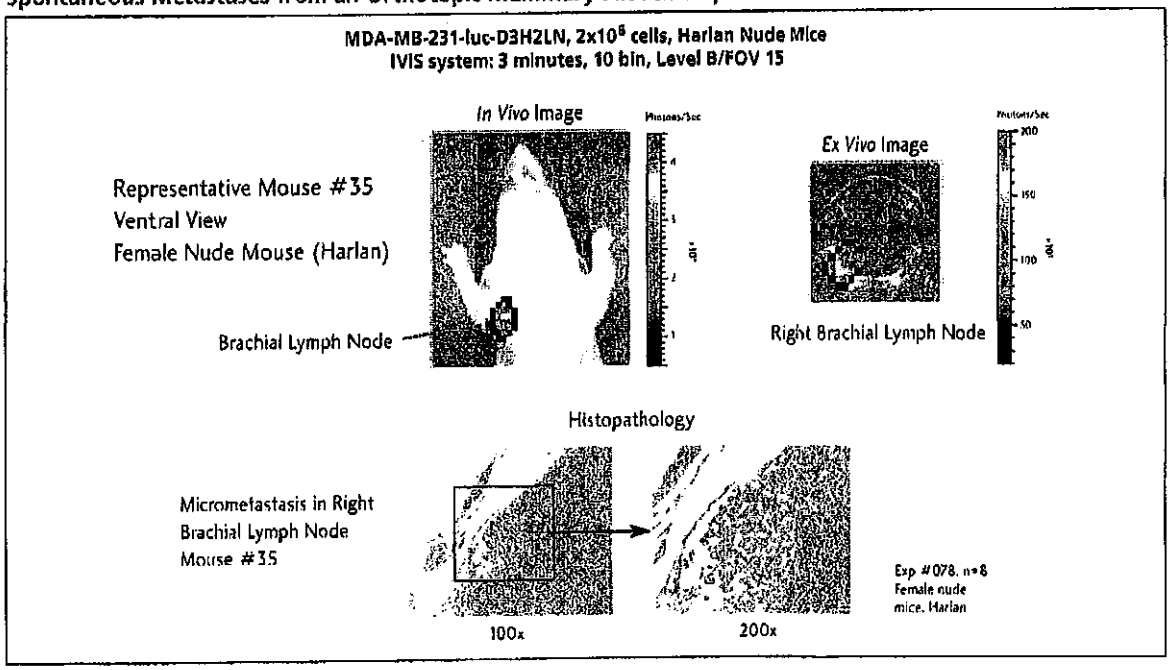
Orthotopic Mammary Fat Pad Tumor Growth-Nude beige mice (CR)



Protocols:
MDA-MB-231-luc-D3H2LN cells ($2x10^6$) are injected into the mammary fat pad of female nude beige mice (Charles River). Mice are imaged weekly for 9 weeks to monitor tumor growth.

Conclusions:
In vivo imaging demonstrates the progression of MDA-MB-231-luc tumors in the mammary fat pad of female nude beige mice (Charles River). Correlation of mean tumor volume to mean bioluminscence is R2=0.958

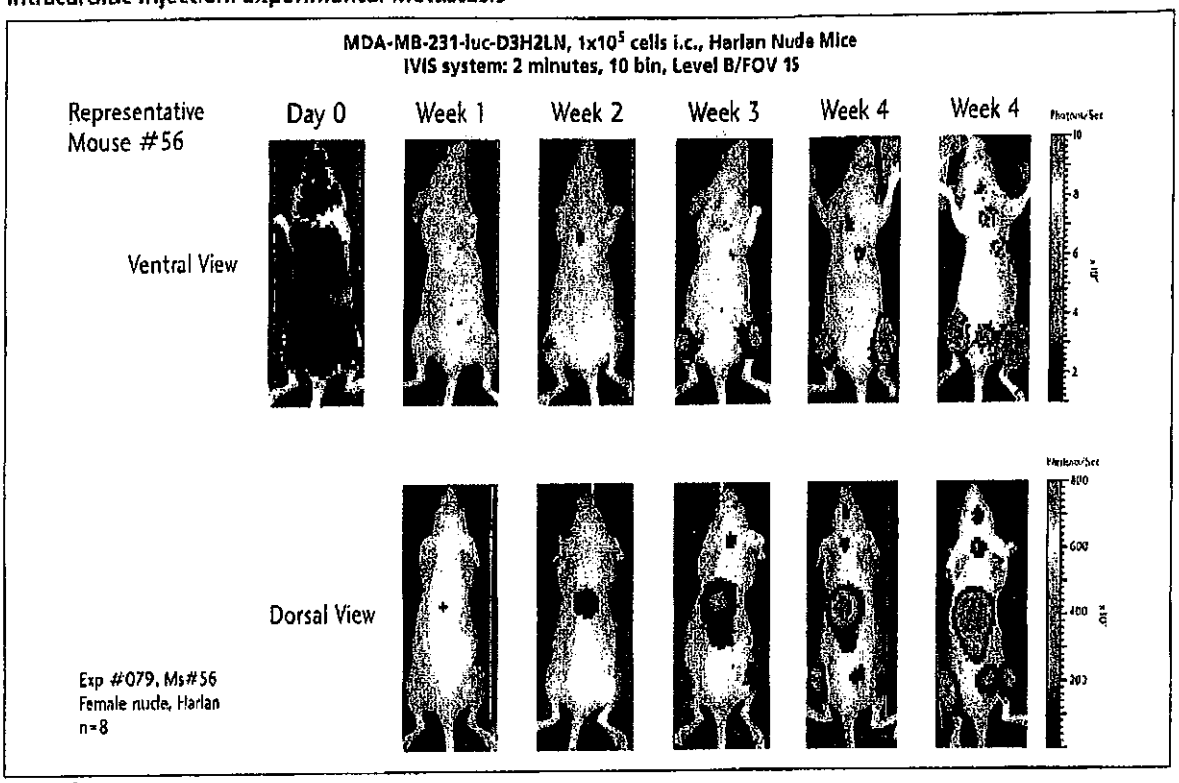
Spontaneous Metastases from an Orthotopic Mammary Fat Pad Implant



Protocols:
MDA-MB-231-luc-D3H2LN cells ($2x10^6$) are injected orthotopically into the abdominal mammary fat pad of female nude mice (Harlan) (n=8). Mice are imaged weekly for five weeks from the ventral view. Primary tumors are shielded in order to detect low signals from secondary metastases. Selected tissues are analyzed by ex vivo imaging and processed for subsequent histology.

Conclusions:
Metastatic signals begin to appear after 3-4 weeks. By week 4, lymph node metastases were detected in vivo in 100% (8/8) of mice. Subsequent histopathology confirmed metastases in 5/8 lymph nodes.

Intracardiac Injection: Experimental Metastasis



Protocols:
MDA-MB-231-luc-D3H2LN cells (1x10^5) were injected into the left ventricle of female nude mice (n=8). Mice were imaged weekly from dorsal and ventral views for 5 weeks. Selected tissues were imaged ex vivo to confirm in vivo signals.

Conclusions:
Metastatic signals begin to appear after 2 weeks. By week 3, metastases were detected in vivo in 100% of mice (8/8) to multiple sites.

Contact Information:

If you have any questions regarding these cell lines, please contact Caliper at 508-497-6592 or e-mail: reagents@caliperls.com

www.caliperLS.com

References

1. Contag CH, Jenkins DE, Contag PR, Negrin R. Use of Reporter Genes for Optical Measurements of Neoplastic Disease *In Vivo*. *Neoplasia* 2000 Jan-Apr;2(1-2):41-52.
2. Edinger M, Cao YA, Hornig YS, Jenkins DE, Verneris MR, Bachmann MH, Negrin RS, Contag CH. Advancing animal models of neoplasia through *in vivo* bioluminescence imaging. *Eur J Cancer* 2002 Nov;38(16):2128-36.
3. Jenkins DE, Oei Y, Hornig Y, Yu SF, Dusich J, Purchio T, Contag PR. Bioluminescent Imaging (BLI) to Improve and Refine Traditional Murine Models of Tumor Growth and Metastasis. *Clin Exp Metastasis* 2003;20(8):733-44.
4. Jenkins DE, Yu SF, Hornig YS, Purchio T, Contag PR. In Vivo Monitoring of Tumor Relapse and Metastasis Using Bioluminescent PC-3M-luc-C6 cells in Murine Models of Human Prostate Cancer. *Clin Exp Metastasis* 2003;20(8):745-56.
5. Murray LJ, Abrams TJ, Long KR, Ngai TJ, Olson LM, Hong W, Keast PK, Brassard JA, O'Farrell AM, Cherrington JM, Pryer NK. SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. *Clin Exp Metastasis* 2003;20(8):757-66.
6. Mendel DB et al. In vivo antitumor activity of SU11248, a novel tyrosine kinase inhibitor targeting vascular endothelial growth factor and platelet-derived growth factor receptors: determination of a pharmacokinetic/pharmacodynamic relationship. *Clin Cancer Res*. 2003 Jan; 9(1):327-37.
7. Scatena CD, Hepner MA, Oei YA, Dusich JM, Yu SF, Purchio T, Contag PR, Jenkins DE. Imaging of Bioluminescent LNCaP-luc-M6 Tumors: A New Animal Model for the Study of Metastatic Human Prostate Cancer. *Prostate* 2004 May 15;59(3): 292-303 .
8. Jenkins DE, Hornig Y, Oei Y, Dusich J, Purchio T. Bioluminescent human breast cancer cell lines that permit rapid and sensitive *in vivo* detection of mammary tumors and multiple metastases in immune deficient mice. *Breast Cancer Research* 2005, 7:R444-R454 (8 April 2005)

Credits

Bioware cell line information compiled by Yvette Hornig, Joan Dusich and Darlene Jenkins. Edited by Joycelyn Bishop.

Disclaimer

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Under the terms of Promega license, the use of this product and derivatives thereof is strictly limited to in vivo research use. Researchers may use this product in their research and they may transfer derivatives to others for research use provided at the time of transfer a copy of this label license is given to the recipients and the recipients agree to be bound by the terms and conditions of this label license. In addition, researchers performing in vivo bioluminescent imaging must do one of the following: (1) use luminescent assay reagents purchased from Promega or Caliper Life Sciences for all determinations of luminescent activity resulting from the research use of this product and its derivatives; or (2) contact Promega to obtain a license for the use of the product and its derivatives in conjunction with luminescent assay reagents not purchased from Promega or Caliper Life Sciences. No reach-through payments shall be owed to Promega relating to an organization's commercialization of products that are discoveries resulting from the research use of this product or its derivatives, provided that such products of the organization do not fall within the valid claims of any issued patents assigned to or licensed by Promega, or that such commercialization would not be a violation of the terms of this label license. No right to use this product for any diagnostic, therapeutic, or commercial application is hereby conveyed to the purchaser of this product. Commercial application is defined as any and all uses of this product and derivatives by a party for monetary or other consideration and may include but is not limited to use in: (1) product manufacture; or (2) to provide a service, information or data; and/or (3) resale of the product or its derivatives, whether or not such product or its derivatives are sold for use in research. If the purchaser is not willing to accept the conditions of this limited label license, Caliper Life Sciences is willing to accept the return of the unopened product and provide the purchaser with a full refund. However, in the event the product is opened, then the purchaser agrees to be bound by the conditions of this limited label license.

Buyer acknowledges that the wild-type and mutant recombinant Coleoptera luciferase nucleic acid and protein ("UC Patented Material") is the subject of U.S. Patent Nos. 5,583,024, 5,674,713 and 5,700,673, assigned to The Regents of The University of California.

The UC Patented Material, or any material that contains or incorporates the UC Patented Material, may not be transferred or licensed to any other party, or be used for commercial purposes by any other party, without a commercial license or the express written consent of The Regents of The University of California.

The Materials may be used solely for internal research, and no right to use the Materials for any human in vivo, diagnostic, therapeutic, or commercial application is hereby conveyed to Buyer.

Additionally, the Buyer may not:

(1) use the Materials in the course of providing a service or data to third parties;

(2) transfer the Materials to any third party or to any place other than Buyer's premises;

(3) attempt to alter, modify or re-engineer the Materials in any way, or extract or transfer any genetic material from the product to another organism; or

(4) sublicense the rights granted herein.

- Buyer is not prohibited from using the Materials to discover or develop products that it intends to sell for consideration as long as those products do not contain any Materials.
- If Buyer has purchased breeding rights to an animal model, Buyer may have the animal model bred by a third party, provided that such third party

  (i) does not use the animal model for any purpose other than breeding for the benefit of Buyer,

  (ii) destroys or returns the animals upon conclusion of the breeding services, and

  (iii) is otherwise legally bound by the terms of this label license.
- Buyer agrees that the Materials are and shall be owned and/or controlled by Caliper Life Sciences, not by Buyer, and that these terms and conditions create a bailment with Buyer with respect to any and all such Materials.
- Buyer agrees that all intellectual property rights relating to the Materials (including, but not limited to, those rights concerning the composition, methods of production, or uses, of the Materials) are and shall continue to be owned and/or controlled by Caliper Life Sciences and not by Buyer.
- However, Caliper Life Sciences shall not claim ownership of any invention made by Buyer using the Materials but which relates to subject matter other than the Materials.
- Buyer may terminate this bailment at any time upon written notice, and Caliper Life Sciences may terminate this bailment in the event of breach of this label license by Buyer.
- In the event of termination hereof, Buyer shall destroy or return all Materials to Caliper Life Sciences.

Caliper LifeSciences
Corporate Headquarters
68 Elm Street
Hopkinton, MA 01748-1668
Tel: 1.508.435.9500
Email: cust.support@caliperLS.com
www.caliperLS.com

Section 3.2

Human Source Material

Office of Research Ethics
The University of Western Ontario
Room 5150 Support Services Building, London, ON, Canada N6A 3K7
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. M. Brackstone
Review Level: Full Board
Review Number: 15925
Revision Number:
Review Date: February 08, 2011
Approved Local # of Participants: 52
Protocol Title: Multimodal Evaluation of Individual Response to Neoadjuvant Chemotherapy/Radiation in Locally Advanced Breast Cancer
Department and Institution: Surgical Oncology, London Health Sciences Centre
Sponsor: INTERNAL RESEARCH FUND-HOSPITAL
Ethics Approval Date: February 08, 2011
Expiry Date: December 31, 2019
Documents Reviewed and Approved: Updated Approval
Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Janice Sutherland

Chair of HSREB: Dr. Joseph Gilbert
FDA Ref. #: IRB 00000940

Ethics Officer to Contact for Further Information			
☑ Janice Sutherland (jsuther@uwo.ca)	☐ Elizabeth Wambolt (ewambolt@uwo.ca)	☐ Grace Kelly (grace.kelly@uwo.ca)	

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cc: ORE File
LHRI

Section 4.2

Plasmid List

Gene Transformed/Transfected

And

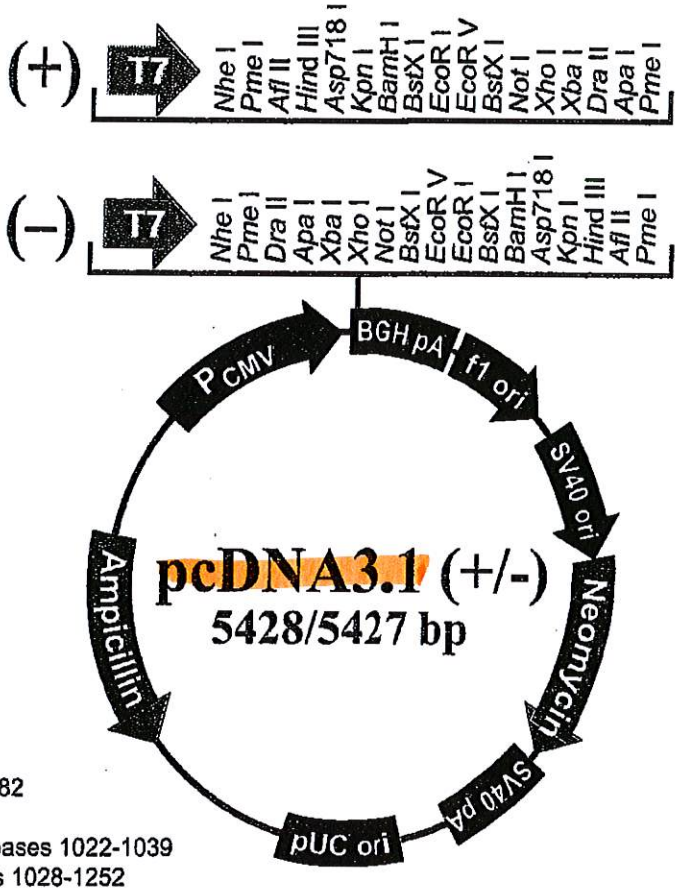
MSDS

Biological Agents Registry Form
Jan. 2012

4.2 Genetic Modifications involving plasmids

Plasmid	Supplier	Gene Inserted	Publication Link
pcDNA3.1(+)	Invitrogen	hOPNa hOPNb hOPNc	
pcDNA3.1(+)/myc-His B	Invitrogen	VANGL1 WNT5A	
pZsGreen1-C1	Clontech	TBX3 iso1 TBX3 iso2	
pSuper.puro	Dr. Shevde	shRNA NM636 shRNA scrambled	PMID:16830233 PMID:16830233
pGEX2T	GE Healthcare	hOPNa hOPNb hOPNc	
pLKO.1-puro/shRNA	Dr. J. Moffatt	shLuciferase shWNT5A shVANGL1	
pGIPZ/shS100A2	Dr. M. Golding	shS100A2	

Invitrogen™
life technologies



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 350484
Product name pcDNA3.1/(+)

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

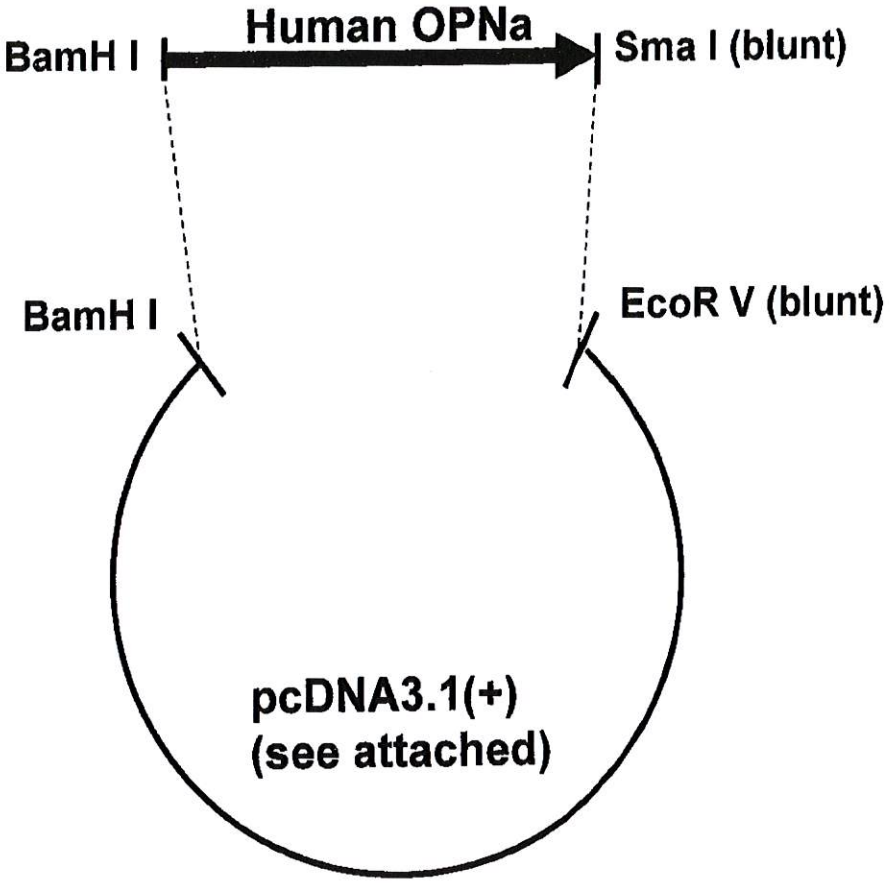
16. OTHER INFORMATION

For research use only

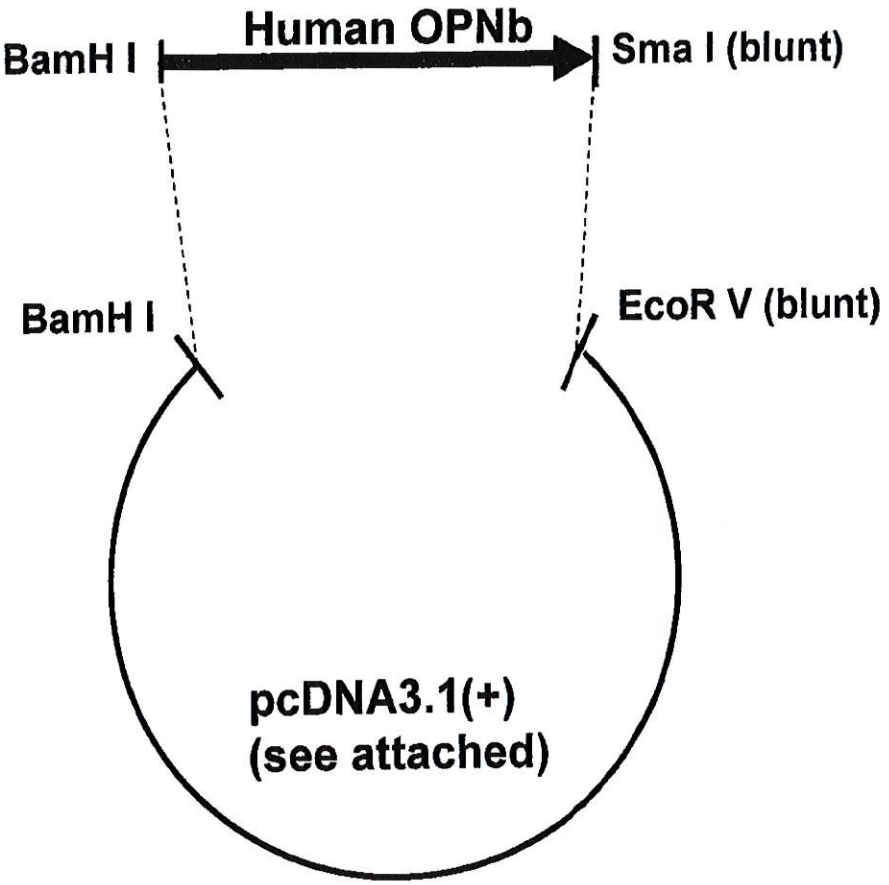
The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

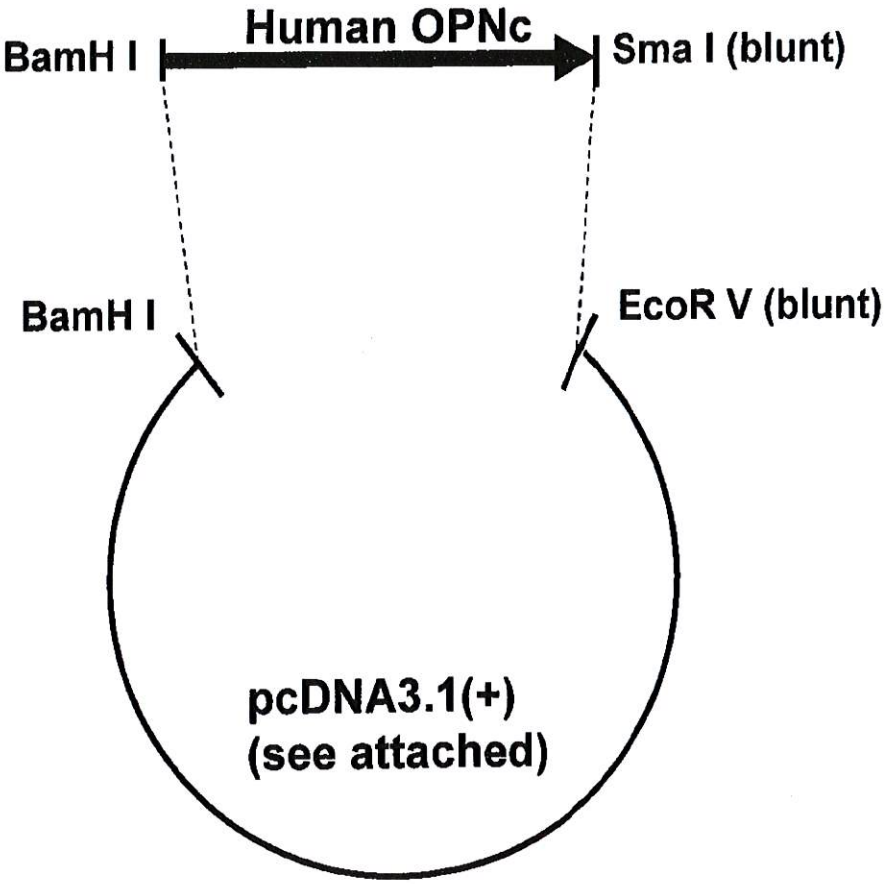
Plasmid: pcDNA3-hOPNa



Plasmid: pcDNA3-hOPNb



Plasmid: pcDNA3-hOPNc

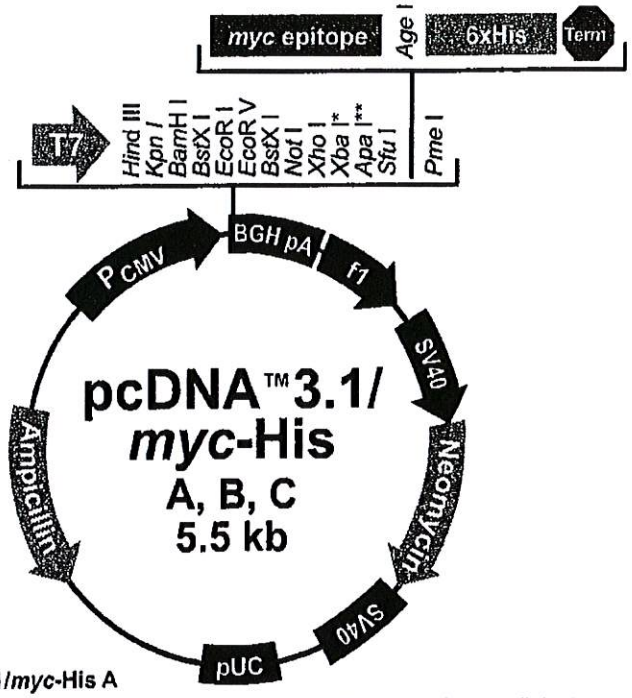


Appendix

pcDNA™3.1/*myc*-His A, B, and C

Map of pcDNA™3.1/*myc*-His

The figure below summarizes the features of the pcDNA™3.1/*myc*-His vectors. The nucleotide sequence for pcDNA™3.1/*myc*-His A is available for downloading from www.invitrogen.com or from **Technical Support** (page 11). Details of the multiple cloning sites for pcDNA™3.1/*myc*-His A, B, and C are shown on pages 3–4.

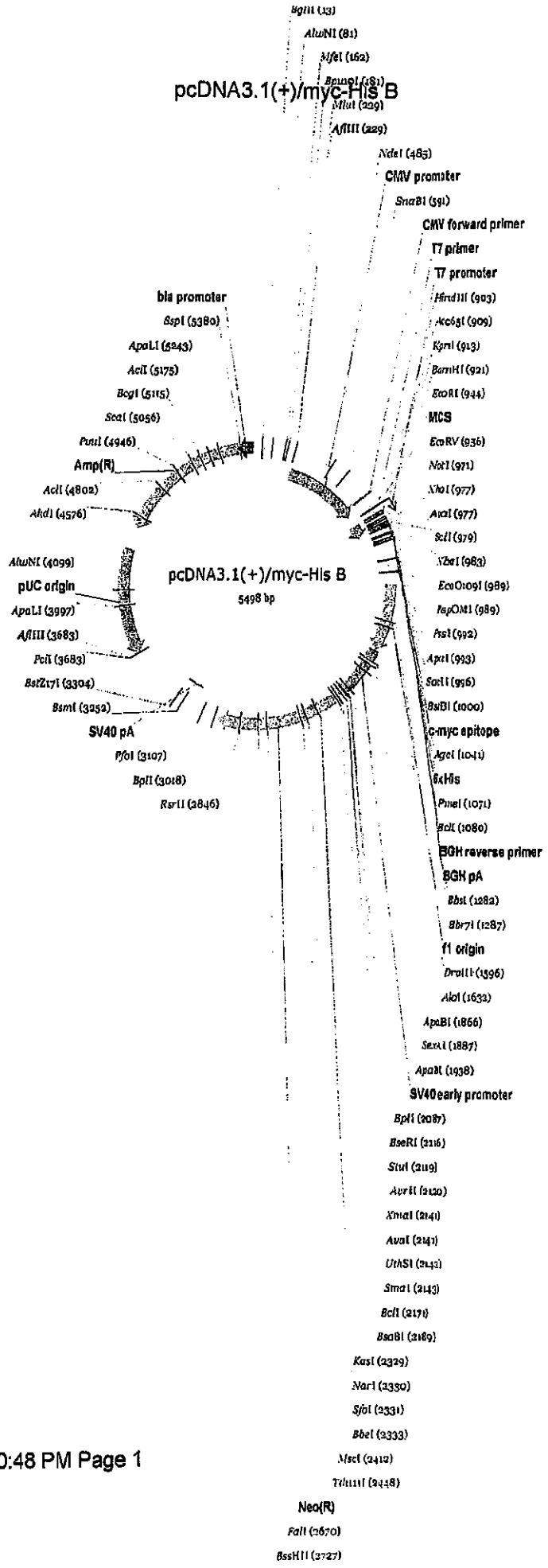


Comments for pcDNA™3.1/*myc*-His A
5493 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
myc epitope: bases 997-1026
Polyhistidine tag: bases 1042-1059
BGH reverse priming site: bases 1082-1099
BGH polyadenylation signal: bases 1081-1295
f1 origin of replication: bases 1358-1771
SV40 promoter and origin: bases 1836-2160
Neomycin resistance gene: bases 2196-2990
SV40 polyadenylation signal: bases 3166-3296
pUC origin: bases 3679-4352
Ampicillin resistance gene: bases 4497-5357 (complementary strand)

* There is a unique *BstE* II site, but no *Xba* I or *Apa* I sites in version C.

** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.


pcDNA3.1(+)/myc-His B
5498 bp
BglII (13)
AlwNI (81)
MfeI (162)
BsmBI (181)
MluI (229)
AflIII (229)
NdeI (485)
CMV promoter
SnaBI (591)
CMV forward primer
T7 primer
T7 promoter
HindIII (903)
Acc65I (909)
KpnI (913)
BamHI (921)
EcoRI (944)
MCS
EcoRV (936)
NotI (971)
XhoI (977)
AvaI (977)
SclI (979)
XbaI (983)
Eco0109I (989)
BspOM1 (989)
PstI (992)
ApaI (993)
SacII (996)
BsiWI (1000)
c-myc epitope
AgeI (1041)
6xHis
PmeI (1071)
BglI (1080)
BGH reverse primer
BGH pA
BbsI (1282)
BbrPI (1287)
f1 origin
DraIII (1396)
AloI (1632)
ApaBI (1866)
SexAI (1887)
ApaBI (1938)
SV40 early promoter
BplI (2087)
BseRI (2116)
StuI (2119)
AvrII (2120)
XmaI (2141)
AvaI (2141)
UthSI (2142)
SmaI (2143)
BclI (2171)
BsaBI (2189)
KasI (2329)
NarI (2330)
SfoI (2331)
BbeI (2333)
MscI (2412)
TthIIII (2448)
Neo(R)
FalI (2670)
BssHII (2727)
bla promoter
SspI (5380)
ApaLI (5243)
AclI (5175)
BcgI (5115)
ScaI (5056)
PmlI (4946)
Amp(R)
AclI (4802)
AhdI (4576)
AlwNI (4099)
pUC origin
ApaLI (3997)
AflIII (3683)
PciI (3683)
BstZ17I (3304)
BsmI (3252)
SV40 pA
PflOI (3107)
BplI (3018)
RsrII (2846)

invitrogen™

Material Safety Data Sheet

Revision Date: 26-Mar-2009

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 350719
Product name PCDNA3.1/MYC-HIS B 20UG

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Solid

Principle Routes of Exposure/ Potential Health effects

Eyes May cause eye irritation with susceptible persons.
Skin No information available

3. HAZARDS IDENTIFICATION

Inhalation	May cause irritation of respiratory tract.
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures	Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.

Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Solid

Important Health Safety and Environmental Information

Boiling point/range	°C No data available	°F No data available
Melting point/range	°C No data available	°F No data available
Flash point	°C No data available	°F No data available
Autoignition temperature	°C No data available	°F No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	No information available
Inhalation	May cause irritation of respiratory tract.
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

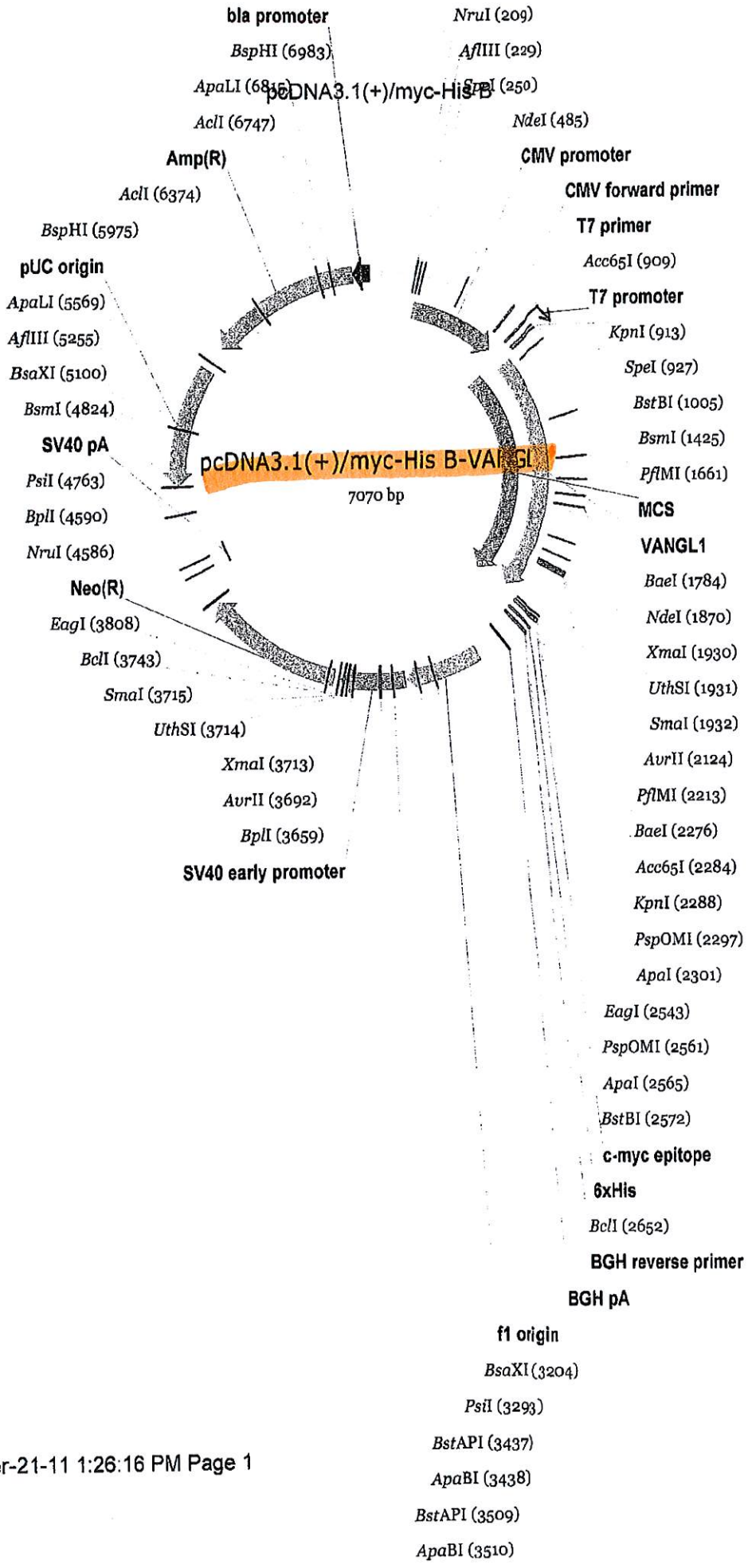
This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

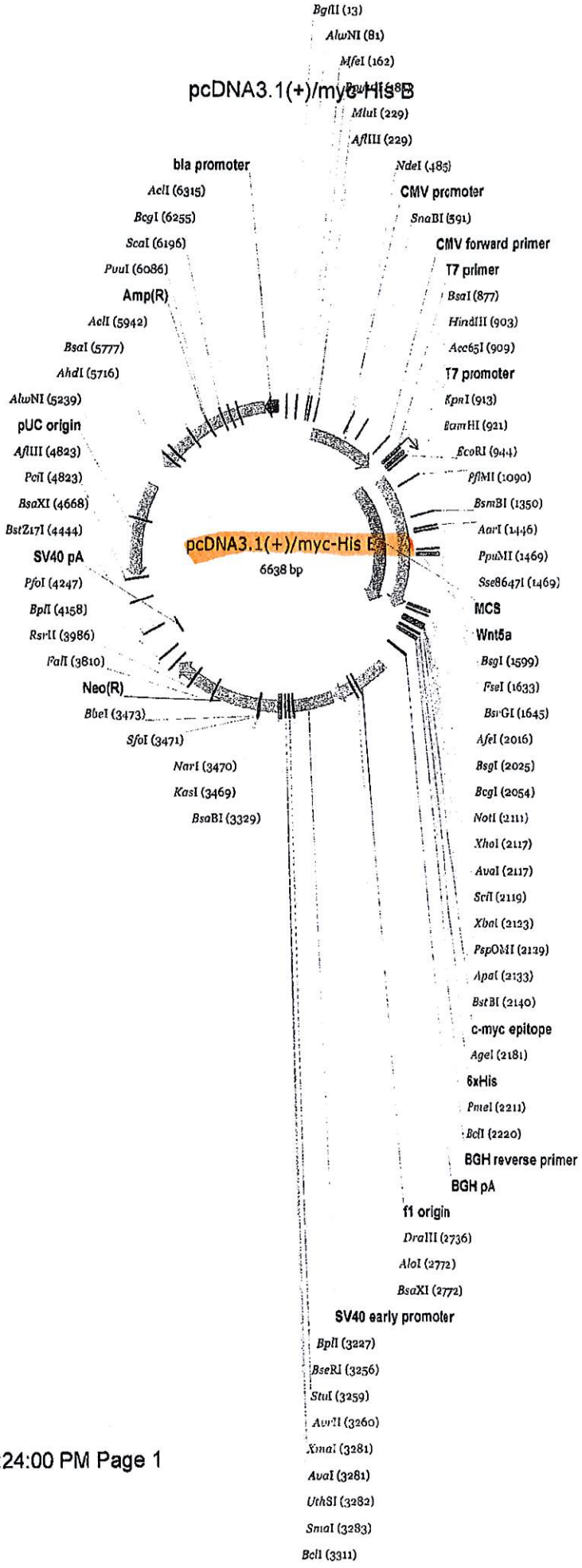
16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet


pcDNA3.1(+)/myc-His B
pcDNA3.1(+)/myc-His B-VANGL
7070 bp
bla promoter
BspHI (6983)
ApaLI (6816)
AclI (6747)
Amp(R)
AclI (6374)
BspHI (5975)
pUC origin
ApaLI (5569)
AflIII (5255)
BsaXI (5100)
BsmI (4824)
SV40 pA
PsiI (4763)
BplI (4590)
NruI (4586)
Neo(R)
EagI (3808)
BclI (3743)
SmaI (3715)
UthSI (3714)
XmaI (3713)
AvrII (3692)
BplI (3659)
SV40 early promoter
NruI (209)
AflIII (229)
SpeI (250)
NdeI (485)
CMV promoter
CMV forward primer
T7 primer
Acc65I (909)
T7 promoter
KpnI (913)
SpeI (927)
BstBI (1005)
BsmI (1425)
PflMI (1661)
MCS
VANGL1
BaeI (1784)
NdeI (1870)
XmaI (1930)
UthSI (1931)
SmaI (1932)
AvrII (2124)
PflMI (2213)
BaeI (2276)
Acc65I (2284)
KpnI (2288)
PspOMI (2297)
ApaI (2301)
EagI (2543)
PspOMI (2561)
ApaI (2565)
BstBI (2572)
c-myc epitope
6xHis
BclI (2652)
BGH reverse primer
BGH pA
f1 origin
BsaXI (3204)
PsiI (3293)
BstAPI (3437)
ApaBI (3438)
BstAPI (3509)
ApaBI (3510)

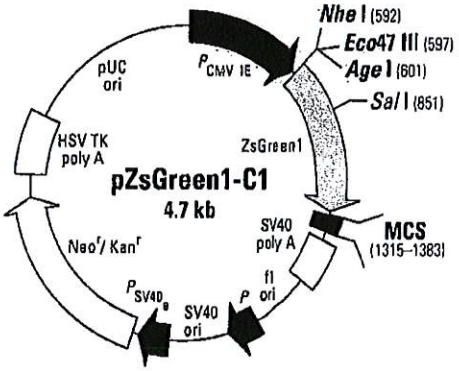

pcDNA3.1(+)/myc-His B
pcDNA3.1(+)/myc-His B
6638 bp
BglII (13)
AlwNI (81)
MfeI (162)
MluI (229)
AflIII (229)
NdeI (485)
CMV promoter
SnaBI (591)
CMV forward primer
T7 primer
BsaI (877)
HindIII (903)
Acc65I (909)
T7 promoter
KpnI (913)
BamHI (921)
EcoRI (944)
PflMI (1090)
BsmBI (1350)
AarI (1446)
PpuMI (1469)
Sse8647I (1469)
MCS
Wnt5a
BsgI (1599)
FseI (1633)
BsrGI (1645)
AfeI (2016)
BsgI (2025)
BcgI (2054)
NotI (2111)
XhoI (2117)
AvaI (2117)
SciI (2119)
XbaI (2123)
PspOMI (2129)
ApaI (2133)
BstBI (2140)
c-myc epitope
AgeI (2181)
6xHis
PmeI (2211)
BclI (2220)
BGH reverse primer
BGH pA
f1 origin
DraIII (2736)
AloI (2772)
BsaXI (2772)
SV40 early promoter
BplI (3227)
BseRI (3256)
StuI (3259)
AvrII (3260)
XmaI (3281)
AvaI (3281)
UthSI (3282)
SmaI (3283)
BclI (3311)
bla promoter
AclI (6315)
BcgI (6255)
ScaI (6196)
PvuI (6086)
Amp(R)
AclI (5942)
BsaI (5777)
AhdI (5716)
AlwNI (5239)
pUC origin
AflIII (4823)
PciI (4823)
BsaXI (4668)
BstZ17I (4444)
SV40 pA
PfoI (4247)
BplI (4158)
RsrII (3986)
PalI (3810)
Neo(R)
BbeI (3473)
SfoI (3471)
NarI (3470)
KasI (3469)
BsaBI (3329)

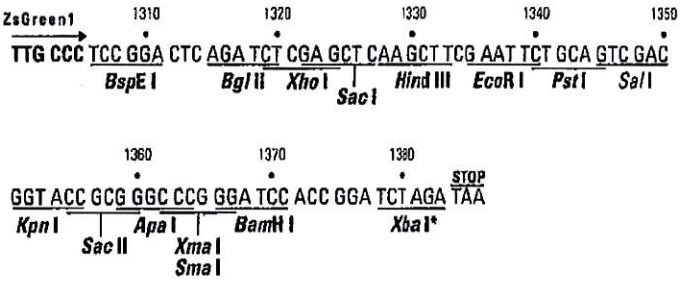
Vector Information

pZsGreen1-C1 Vector Information

PT3487-5

Cat. No. 632447 and sold as part of Cat. No. 630050





Restriction Map and Multiple Cloning Site (MCS) of pZsGreen1-C1. All sites shown are unique. The *Xba* I site (*) is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pZsGreen1-C1 encodes a human codon-optimized variant of wild-type *Zoanthus* sp. green fluorescent protein, ZsGreen1 (1). The ZsGreen1 coding sequence contains a series of silent base-pair changes, which correspond to human codon-usage preferences, for optimal expression in mammalian cells (2). Additionally, an upstream sequence—located just 5' to the ZsGreen1 start codon—has been converted to a Kozak consensus translation initiation site (3) to further increase the translation efficiency in eukaryotic cells. A single amino acid substitution (Asn-65 to Met) has been made to enhance the emission characteristics of ZsGreen1 (excitation maximum = 496 nm; emission maximum = 506 nm).

The multiple cloning site (MCS) in pZsGreen1-C1 is positioned between the ZsGreen1 coding sequence and a pair of SV40 polyadenylation signals (SV40 poly A). Thus, genes cloned into the MCS will be expressed as fusions to the C-terminus of ZsGreen1 if they are in the same reading frame as ZsGreen1 and there are no intervening stop codons. Expression of ZsGreen1 is driven by the cytomegalovirus immediate-early promoter ($P_{CMV\ IE}$). The SV40 poly A signals downstream of the MCS direct proper processing of the 3' end of ZsGreen1 mRNA.

The vector backbone contains an SV40 origin (SV40 ori) for replication in mammalian cells that express the SV40 T-antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A neomycin resistance cassette—consisting of the SV40 early promoter (P_{SV40_E}), the neomycin/kanamycin resistance gene of Tn5 (Neo^r/Kan^r), and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK poly A) gene—allows stably transfected eukaryotic cells to be selected using G418 (4). A bacterial promoter (*P*) upstream of this cassette drives expression of the Neo^r/Kan^r gene in *E. coli* hosts, which can be selected with kanamycin.

(PR641614; published 24 April 2006)

Clontech

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Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

Use

Fusions to the C terminus of ZsGreen1 retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pZsGreen1-C1 so that it is in frame with the ZsGreen1 coding sequence, with no intervening, in-frame stop codons. The recombinant pZsGreen1-C1 vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (available from Clontech; Cat. Nos. 631307 & 631308). We recommend selecting mammalian cell cultures in 500–1,300 µg/ml G418, depending on the cell line. Be sure to establish a kill curve for each cell line and each lot of G418 to determine optimal selection concentration. Unmodified (i.e., non-recombinant) pZsGreen1-C1 can also be used simply to express ZsGreen1 in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  - Enhancer region: 59–465; TATA box: 554–560
  - Transcription start point: 583
  - C→G mutation to remove *Sac* I site: 569
- *Zoanthus* sp. green fluorescent protein (ZsGreen1) coding sequence
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615
  - Asn-65 to Met mutation (A→T, C→G): 809, 810
- Multiple Cloning Site (MCS): 1315–1383
  - Stop codon: 1384–1386
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1526–1531 & 1555–1560; mRNA 3' ends: 1564 & 1576
- f1 single-strand DNA origin: 1623–2078 (Packages the noncoding strand of ZsGreen1.)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2140–2145; –10 region: 2163–2168
  - Transcription start point: 2175
- SV40 origin of replication: 2419–2554
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2252–2323 & 2324–2395
  - 21-bp repeats: 2399–2419, 2420–2440 & 2442–2462
  - Early promoter element: 2475–2481
  - Major transcription start points: 2471, 2509, 2515 & 2520
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2603–2605; stop codon: 3395–3397
  - G→A mutation to remove *Pst* I site: 2785
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3131
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3633–3638 & 3646–3651
- pUC plasmid replication origin: 3982–4625

Propagation in *E. coli*

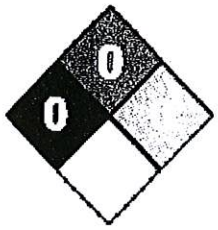
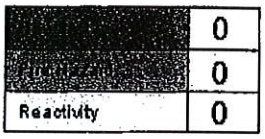
- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (50 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* 17:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* 6:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125–8148.
4. Gorman, C. (1985). In *DNA Cloning: A Practical Approach, Vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech. This vector has not been completely sequenced.

Material Safety Data Sheet

NFPA	HMIS	PPE	Transport Symbol
			

Issuing Date 27-Sep-2006 **Revision Date** **Revision Number** 0

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name pZsGreen1-C1 Vector

Product Code(s) 632447

Supplier Address
Clontech Laboratories, Inc.
1290 Terra Bella
Mountain View, CA 94043
TEL: 650-919-7452

2. HAZARDS IDENTIFICATION

Emergency Overview

The product contains no substances which at their given concentration are considered to be hazardous to health

Appearance White **Physical State** Liquid, frozen. **Odor** Odorless

Potential Health Effects

Acute Toxicity

Eyes	No known effect based on information supplied
Skin	No known effect based on information supplied
Inhalation	No known effect based on information supplied
Ingestion	No known effect based on information supplied

Chronic Effects No known effect based on information supplied

Aggravated Medical Conditions None known

Environmental Hazard See Section 12 for additional Ecological information

3. COMPOSITION/INFORMATION ON INGREDIENTS

Chemical Name	CAS-No	Weight %
Plasmid DNA	Not Applicable	60-100

4. FIRST AID MEASURES

Eye Contact Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician

Skin Contact Wash skin with soap and water

Inhalation Move to fresh air

Ingestion Clean mouth with water and afterwards drink plenty of water

Notes to Physician Treat symptomatically. Materials have been screened to insure they are free of HIV and AIDS.

5. FIRE-FIGHTING MEASURES

Flammable Properties Not flammable

Flash Point No data available

Suitable Extinguishing Media Use extinguishing measures that are appropriate to local circumstances and the surrounding environment

Explosion Data
Sensitivity to mechanical impact None
Sensitivity to static discharge None

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA	**Health Hazard** 0	**Flammability** 0	**Stability** 0	**Physical and Chemical Hazards -**
HMIS	**Health Hazard** 0	**Flammability** 0	**Stability** 0	**Personal Precautions** B

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Avoid contact with skin, eyes and clothing.

Methods for Containment Prevent further leakage or spillage if safe to do so

Methods for Cleaning Up Take up mechanically and collect in suitable container for disposal

7. HANDLING AND STORAGE

Handling Handle in accordance with good industrial hygiene and safety practice. Avoid contact with skin, eyes and clothing.

Storage	Keep in properly labeled containers. Keep container tightly closed. Keep away from heat. Store at -20 Celsius.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Exposure Guidelines	This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.
Engineering Measures	Showers Eyewash stations Ventilation systems
Personal Protective Equipment	
Eye/Face Protection	Safety glasses with side-shields.
Skin and Body protection	Wear protective gloves/clothing.
Respiratory Protection	If exposure limits are exceeded or irritation is experienced, NIOSH/MSHA approved respiratory protection should be worn. Positive-pressure supplied air respirators may be required for high airborne contaminant concentrations. Respiratory protection must be provided in accordance with current local regulations.
Hygiene Measures	Handle in accordance with good industrial hygiene and safety practice

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance	White	**Odor**	Odorless
Odor Threshold	No information available	**Physical State**	Liquid, frozen
pH	No information available		
Flash Point	No data available	**Autoignition Temperature**	Not applicable
Decomposition Temperature	No data available	**Boiling Point/Range**	No data available
Melting Point/Range	No data available		
Flammability Limits in Air	No data available	**Explosion Limits**	No data available
Specific Gravity	No data available	**Solubility**	No data available
Evaporation Rate	No data available	**Vapor Pressure**	No data available
Vapor Density	No data available	**VOC Content**	Not applicable
Partition Coefficient (n-octanol/water)	No data available		

10. STABILITY AND REACTIVITY

Stability	Stable under recommended storage conditions
Conditions to Avoid	Temperatures above -20°C.

Incompatible Products	None known based on information supplied
Hazardous Decomposition Products	None under normal use.
Hazardous Polymerization	Hazardous polymerization does not occur

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information	Product does not present an acute toxicity hazard based on known or supplied information

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product
Reproductive Toxicity	This product does not contain any known or suspected reproductive hazards
Target Organ Effects	None known

12. ECOLOGICAL INFORMATION

Ecotoxicity

Contains no substances known to be hazardous to the environment or that are not degradable in waste water treatment plants

13. DISPOSAL CONSIDERATIONS

Waste Disposal Method	This material, as supplied, is not a hazardous waste according to state and federal regulations (40 CFR 261)
Contaminated Packaging	Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

DOT	Not regulated
TDG	Not regulated
MEX	Not regulated
ICAO	Not regulated
IATA	Not regulated
IMDG/IMO	Not regulated
RID	Not regulated

14. TRANSPORT INFORMATION

ADR Not regulated

ADN Not regulated

15. REGULATORY INFORMATION

International Inventories

TSCA	Exempt
DSL	Does not Comply
EINECS/ELINCS	Does not Comply
ENCS	Does not Comply
CHINA	Does not Comply
KECL	Does not Comply
PICCS	Does not Comply
AICS	Does not Comply

U.S. Federal Regulations

SARA 313
Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazard Categories

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42).

CERCLA
This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material.

U.S. State Regulations

California Proposition 65
This product does not contain any Proposition 65 chemicals.

U.S. State Right-to-Know Regulations

International Regulations

Mexico - Grade Minimum risk, Grade 0

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
Non-controlled

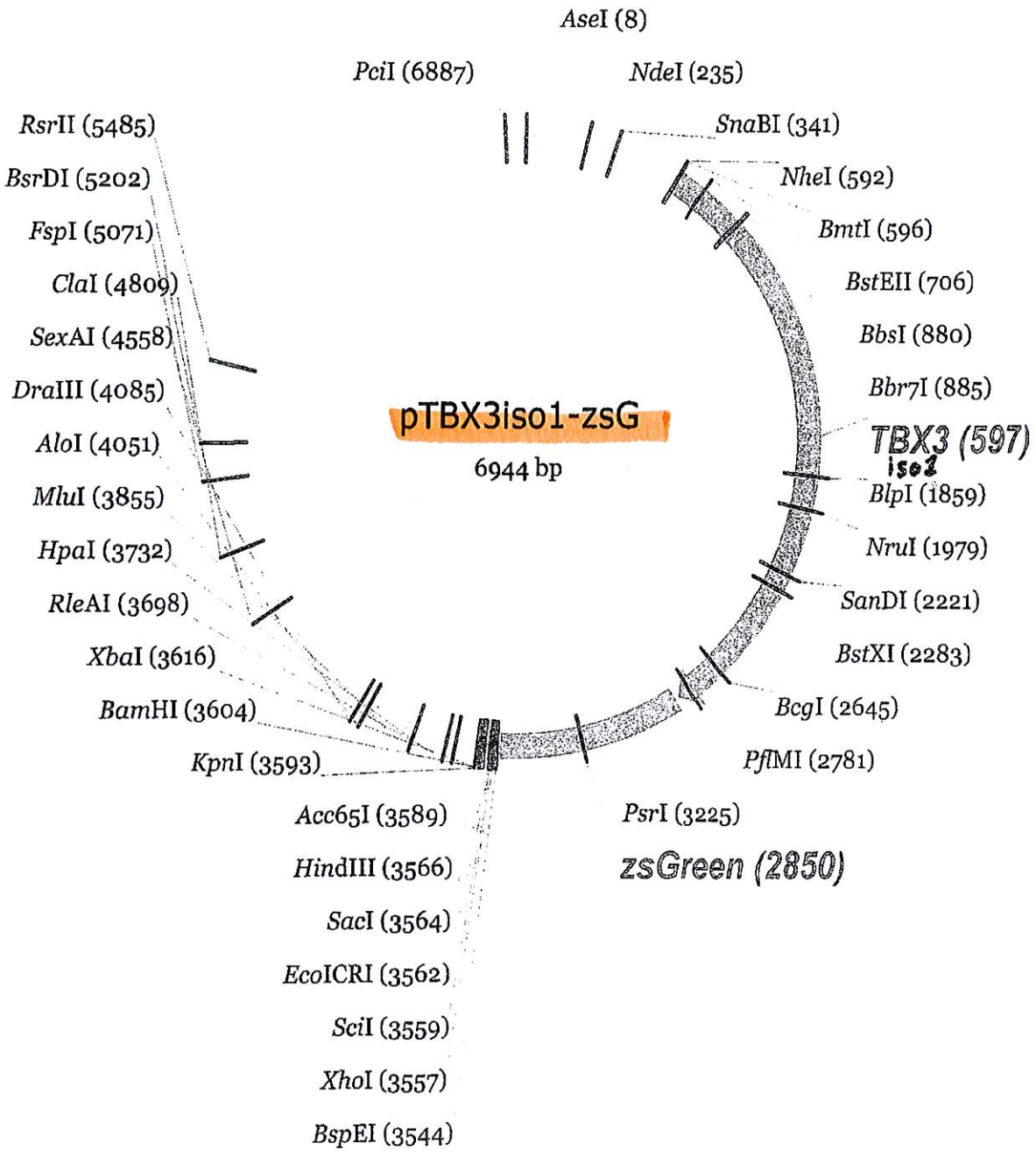
16. OTHER INFORMATION

Issuing Date	27-Sep-2006
Revision Date	
Revision Note	No information available

Disclaimer
The information provided on this MSDS is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

pzsGreen-C1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BmtI (596)
FspI (5071)
BstEII (706)
ClaI (4809)
BbsI (880)
SexAI (4558)
Bbr7I (885)
DraIII (4085)
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TBX3 (597)
iso1
AloI (4051)
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MluI (3855)
NruI (1979)
HpaI (3732)
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RleAI (3698)
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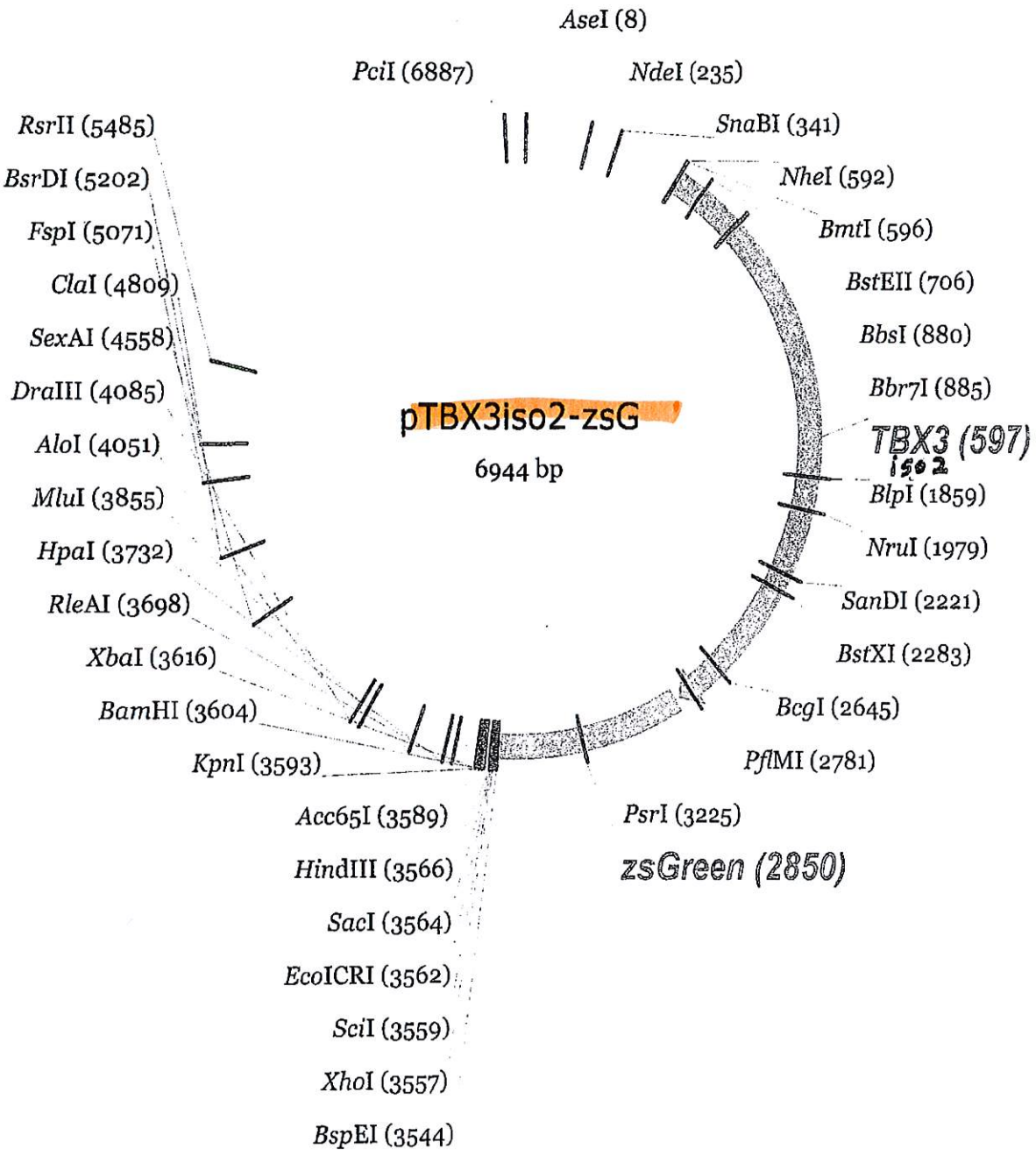
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BbsI (880)
Bbr7I (885)
TBX3 (597)
iso2
BlpI (1859)
NruI (1979)
SanDI (2221)
BstXI (2283)
BcgI (2645)
PflMI (2781)
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Acc65I (3589)
HindIII (3566)
SacI (3564)
EcoICRI (3562)
SciI (3559)
XhoI (3557)
BspEI (3544)
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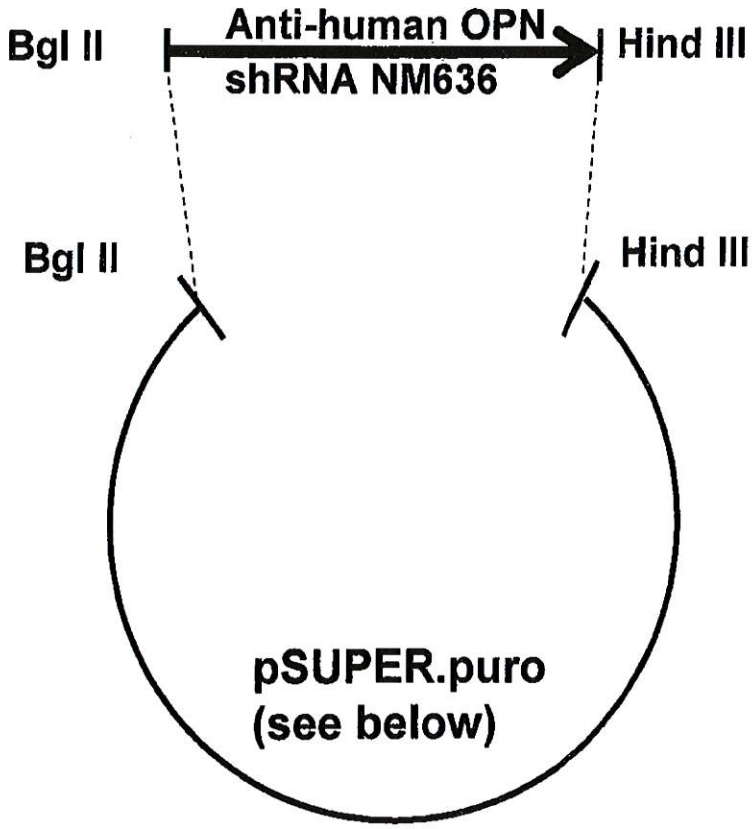
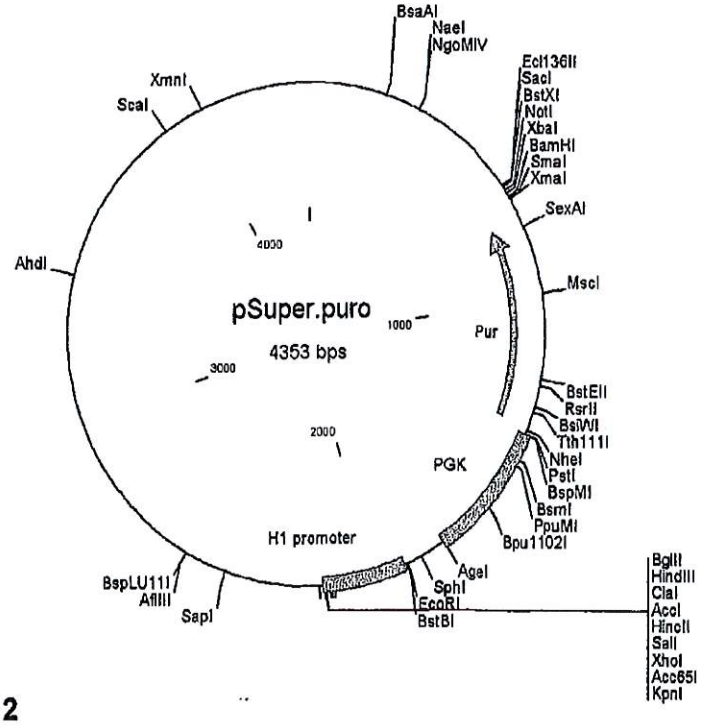
6944 bp

Plasmid: pSUPER-NM636

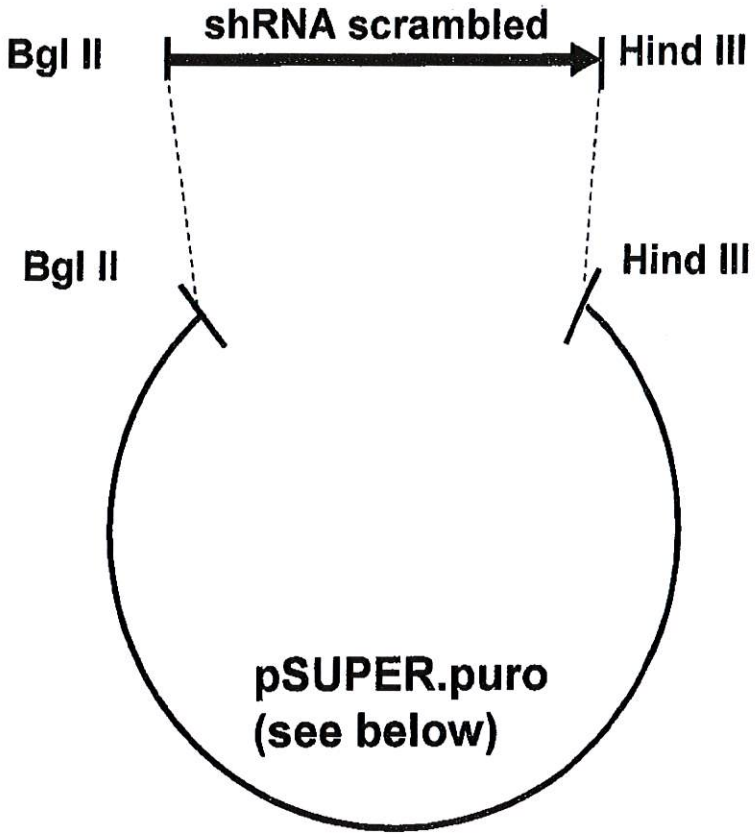
(Shevde et al. (2006) Clin Exp Metastasis. 23:123-33).

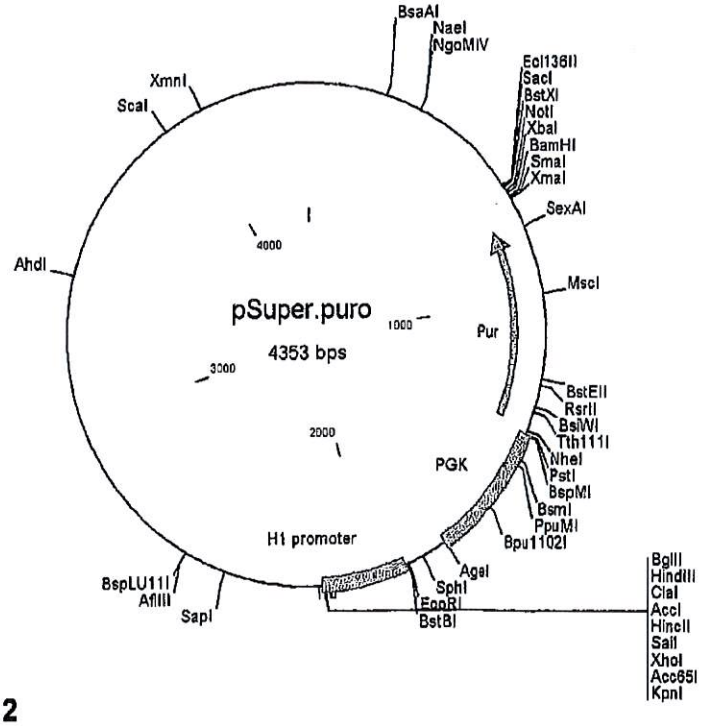




pSUPER.puro from:
Oligoengine
1409 42nd Avenue East
Seattle, Washington 98112

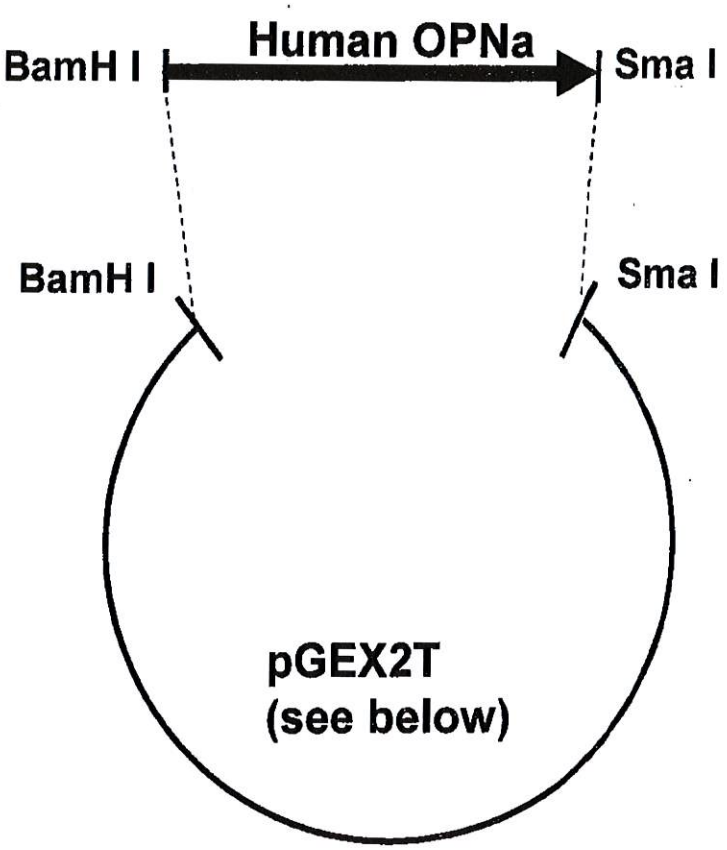
Plasmid: pSUPER-scrambled
(Shevde et al. (2006) Clin Exp Metastasis. 23:123-33).





pSUPER.puro from:
Oligoengine
1409 42nd Avenue East
Seattle, Washington 98112

Plasmid: pGEX2T-hOPNa



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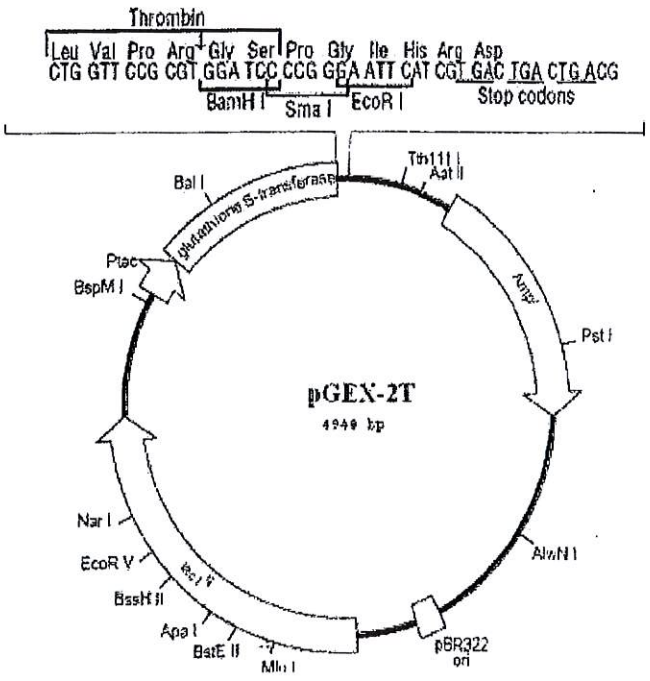
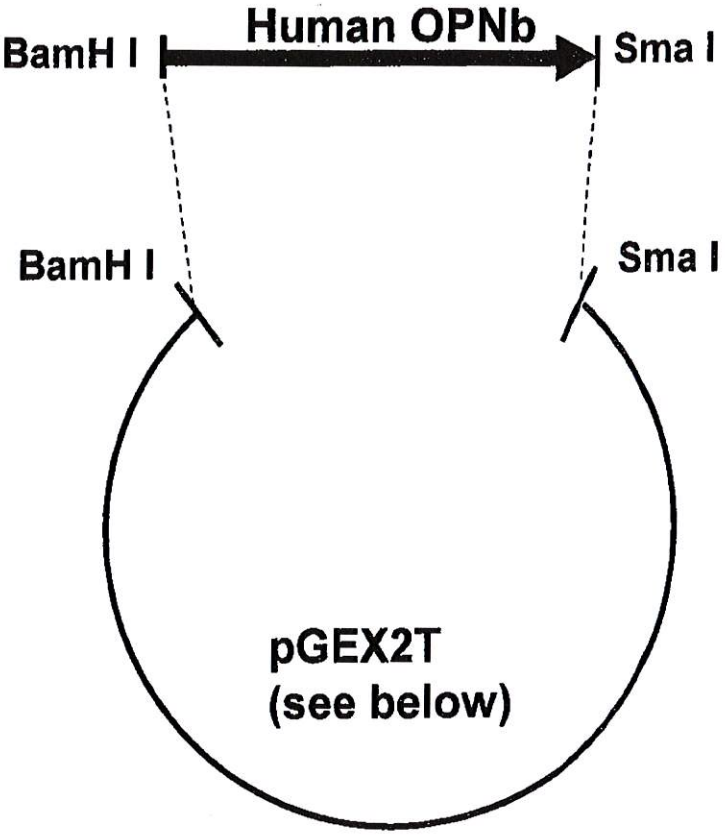


pGEXT2 from:
GE Healthcare
(formerly Amersham)
800 Centennial Avenue
Piscataway, NJ 08855-1327
USA

Plasmid: pGEX2T-hOPNb



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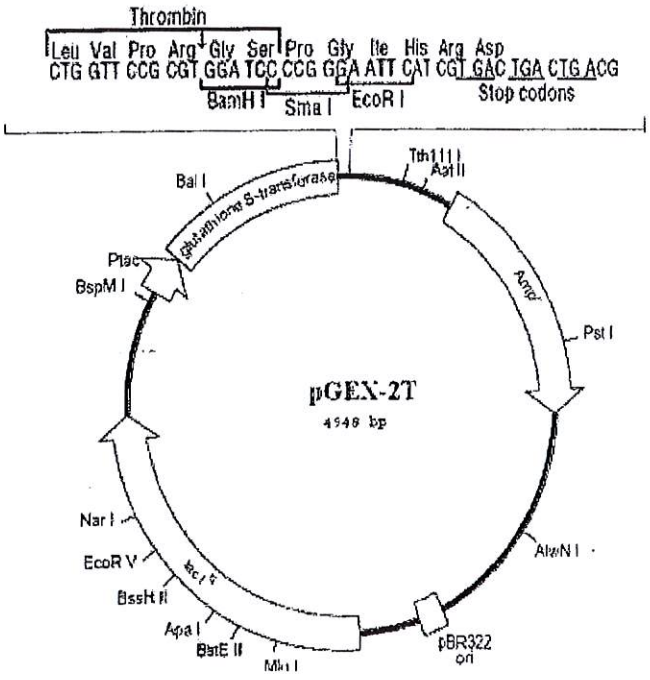

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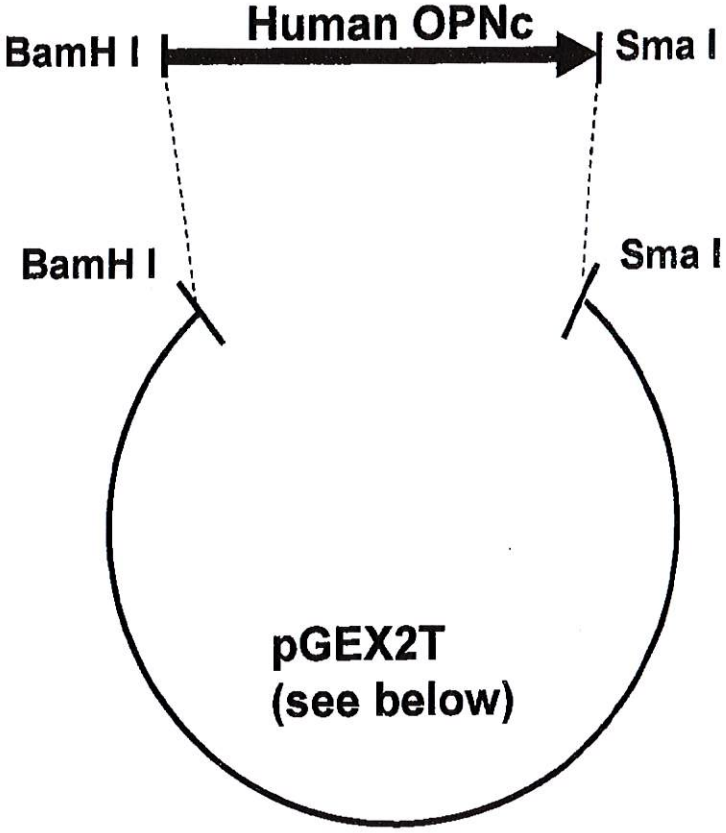
GE Healthcare
(formerly Amersham)
800 Centennial Avenue
Piscataway, NJ 08855-1327
USA

Plasmid: pGEX2T-hOPNc



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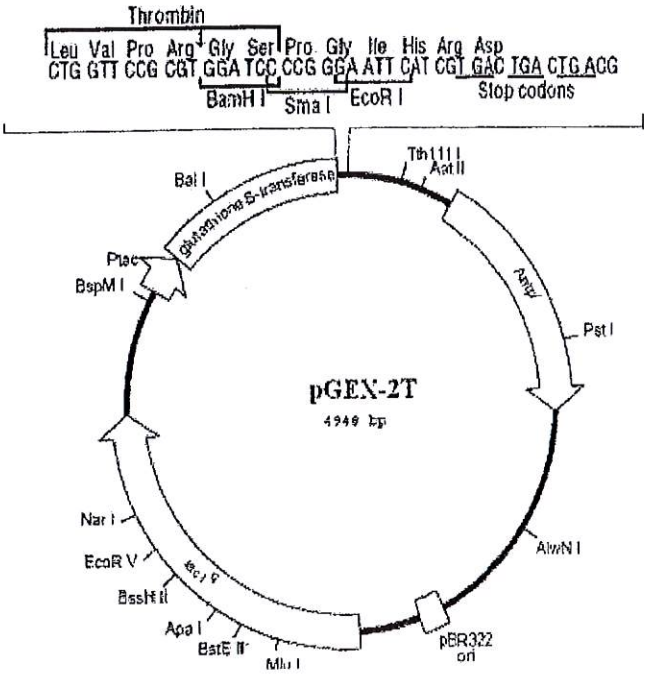

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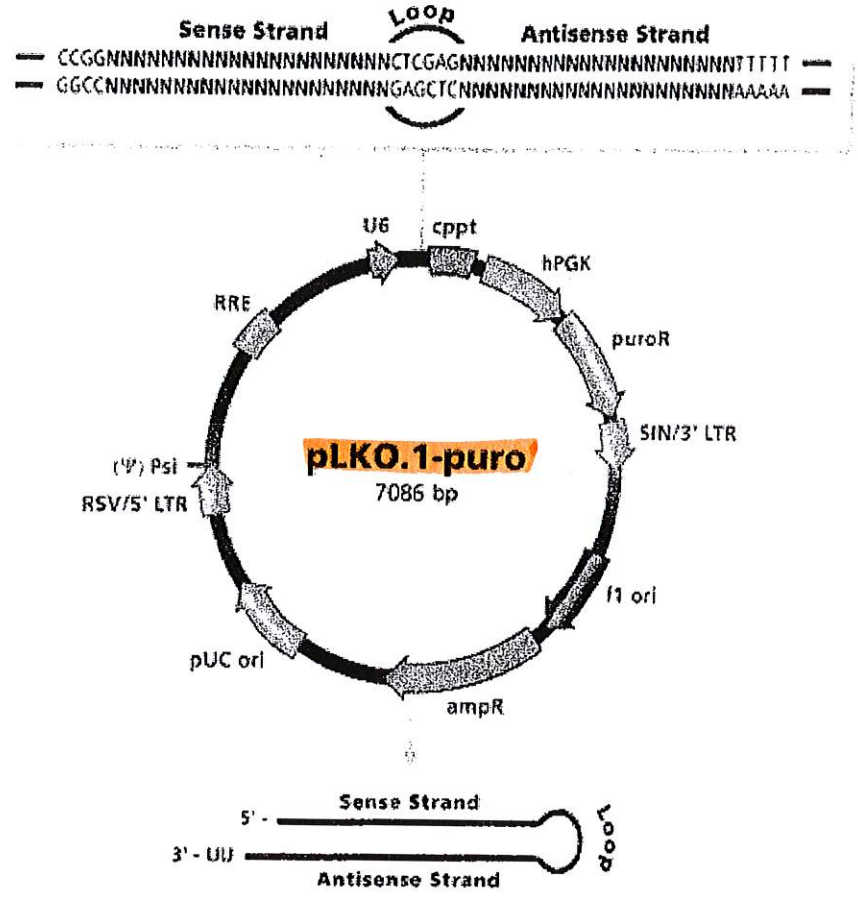
Piscataway, NJ 08855-1327
USA



TRC1.5 Vector Description and Features

Name	Description
cppt	Central polypurine tract
hPGK	Human phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
SIN/LTR	3' self inactivating long terminal repeat
f1 ori	f1 origin of replication
ampR	Ampicillin resistance gene for bacterial selection
pUC ori	pUC origin of replication
5' LTR	5' long terminal repeat
Psi	RNA packaging signal
RRE	Rev response element

back to top

Material Safety Data Sheet

Version 5.1
Revision Date 01/12/2012
Print Date 01/24/2012

1. PRODUCT AND COMPANY IDENTIFICATION

Product name : MISSION® pLKO.1-puro Empty Vector Control Plasmid DNA

Product Number : SHC001
Brand : Sigma
Product Use : For laboratory research purposes.

Supplier : Sigma-Aldrich Canada, Ltd
2149 Winston Park Drive
OAKVILLE ON L6H 6J8
CANADA

Telephone : +1 9058299500
Fax : +1 9058299292
Emergency Phone # (For both supplier and manufacturer) : 1-800-424-9300
Preparation Information : Sigma-Aldrich Corporation
Product Safety - Americas Region
1-800-521-8956

Manufacturer : Sigma-Aldrich Corporation
3050 Spruce St.
St. Louis, Missouri 63103
USA

2. HAZARDS IDENTIFICATION

Emergency Overview

WHMIS Classification

Not WHMIS controlled. Not WHMIS controlled.

Not a dangerous substance or mixture according to the Globally Harmonised System (GHS).

HMIS Classification

Health hazard: 0
Flammability: 0
Physical hazards: 0

Potential Health Effects

Inhalation May be harmful if inhaled. May cause respiratory tract irritation.
Skin May be harmful if absorbed through skin. May cause skin irritation.
Eyes May cause eye irritation.
Ingestion May be harmful if swallowed.

3. COMPOSITION/INFORMATION ON INGREDIENTS

CAS-No.	EC-No.	Index-No.	Concentration
Water			
7732-18-5	231-791-2	-	98.002 %
2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride			
1185-53-1	214-684-5	-	1.576 %
Edetate disodium dihydrate			
6381-92-6	205-358-3	-	0.372 %
Deoxyribonucleic acid			
9007-49-2	-	-	0.05 %

4. FIRST AID MEASURES

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

5. FIREFIGHTING MEASURES

Conditions of flammability
Not flammable or combustible.

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

Special protective equipment for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

Hazardous combustion products
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

Explosion data - sensitivity to mechanical impact
no data available

Explosion data - sensitivity to static discharge
no data available

6. ACCIDENTAL RELEASE MEASURES

Personal precautions
Avoid breathing vapors, mist or gas.

Environmental precautions
Do not let product enter drains.

Methods and materials for containment and cleaning up
Keep in suitable, closed containers for disposal.

7. HANDLING AND STORAGE

Conditions for safe storage
Keep container tightly closed in a dry and well-ventilated place.

Recommended storage temperature: -20 °C

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Contains no substances with occupational exposure limit values.

Personal protective equipment

Respiratory protection
Respiratory protection not required. For nuisance exposures use type OV/AG (US) or type ABEK (EU EN 14387) respirator cartridges. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Hand protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Eye protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin and body protection
Impervious clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Hygiene measures
General industrial hygiene practice.

Specific engineering controls
Use mechanical exhaust or laboratory fumehood to avoid exposure.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance

Form	liquid
Colour	no data available

Safety data

pH	no data available
Melting point/freezing point	no data available
Boiling point	no data available
Flash point	no data available
Ignition temperature	no data available
Autoignition temperature	no data available
Lower explosion limit	no data available
Upper explosion limit	no data available
Vapour pressure	no data available
Density	no data available
Water solubility	no data available
Partition coefficient: n-octanol/water	no data available
Relative vapour density	no data available
Odour	no data available
Odour Threshold	no data available
Evaporation rate	no data available

10. STABILITY AND REACTIVITY

Chemical stability
Stable under recommended storage conditions.

Possibility of hazardous reactions
no data available

Conditions to avoid
no data available

Materials to avoid
no data available

Hazardous decomposition products
Other decomposition products - no data available
Hazardous decomposition products formed under fire conditions. - Carbon oxides, nitrogen oxides (NOx), Hydrogen chloride gas

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Oral LD50
no data available

Inhalation LC50
no data available

Dermal LD50
no data available

Other information on acute toxicity
no data available

Skin corrosion/irritation
no data available

Serious eye damage/eye irritation
Eyes: no data available

Respiratory or skin sensitization
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.

Reproductive toxicity

no data available

Teratogenicity

no data available

Specific target organ toxicity - single exposure (Globally Harmonized System)
no data available

Specific target organ toxicity - repeated exposure (Globally Harmonized System)
no data available

Aspiration hazard
no data available

Potential health effects

Inhalation	May be harmful if inhaled. May cause respiratory tract irritation.
Ingestion	May be harmful if swallowed.
Skin	May be harmful if absorbed through skin. May cause skin irritation.
Eyes	May cause eye irritation.

Signs and Symptoms of Exposure
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Synergistic effects
no data available

Additional Information
RTECS: Not available

12. ECOLOGICAL INFORMATION

Toxicity

no data available

Persistence and degradability
no data available

Bioaccumulative potential
no data available

Mobility in soil
no data available

PBT and vPvB assessment
no data available

Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

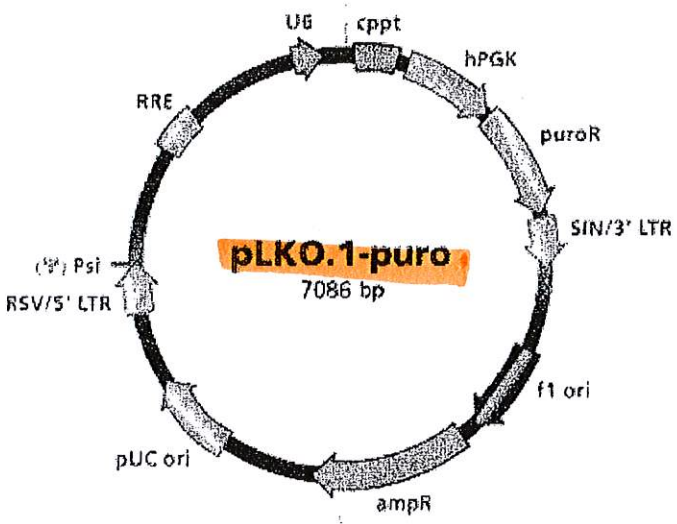
WHMIS Classification
Not WHMIS controlled. Not WHMIS controlled.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

16. OTHER INFORMATION

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.



shRNA genes inserted in vector

a) Luciferase
b) VANGL1
c) WNT5A

TRC1.5 Vector Description and Features

Name	Description
cppt	Central polypurine tract
hPGK	Human phosphoglycerate kinase eukaryotic promoter
puroR	Puromycin resistance gene for mammalian selection
SIN/LTR	3' self inactivating long terminal repeat
f1 ori	f1 origin of replication
ampR	Ampicillin resistance gene for bacterial selection
pUC ori	pUC origin of replication
5' LTR	5' long terminal repeat
Psi	RNA packaging signal
RRE	Rev response element

back to top

Use of the miR30 design also allowed the use of 'rules-based' designs for target sequence selection. One such rule is the destabilizing of the 5' end of the antisense strand which results in strand specific incorporation of miRNAs into RISC.

The proprietary design algorithm targets sequences in coding regions and the 3'UTR with the additional requirement that they contain greater than 3 mismatches to any other sequence in the human or mouse genomes.

Each shRNA construct has been sequence verified to ensure a match to the target gene. To assure you the highest possibility of modulating the gene expression level, each gene is represented by multiple shRNA constructs, each covering a unique region of the target gene.

Vector Information

Versatile Vector Design

Features of the pGIPZ lentiviral vector (Figure 2-3, Table 1) that make it a versatile tool for RNAi studies include:

- Ability to perform transfections or transductions using the replication incompetent lentivirus (Shimada, *et al.* 1995)
- TurboGFP and shRNAmir are part of a bicistronic transcript allowing the visual marking of shRNAmir expressing cells
- Amenable to *in vitro* and *in vivo* applications
- Puromycin drug resistance marker for selecting stable cell lines
- Molecular barcodes enable multiplexed screening in pools

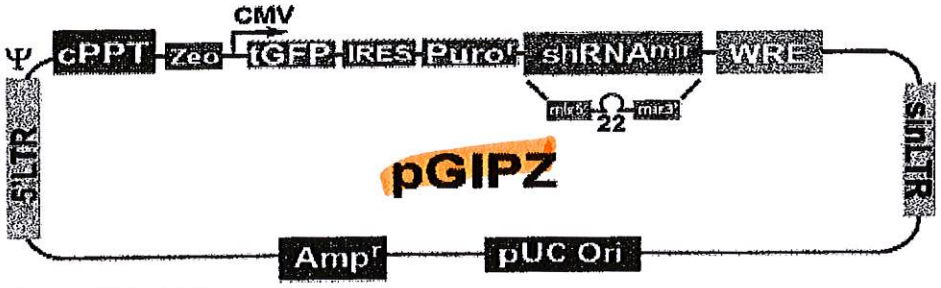


Figure 2. pGIPZ lentiviral vector

Table 1. Features of the pGIPZ vector

Vector Element	Utility
CMV Promoter	RNA Polymerase II promoter
cPPT	Central Polypurine tract helps translocation into the nucleus of non-dividing cells
WRE	Enhances the stability and translation of transcripts
TurboGFP	Marker to track shRNAmir expression
IRES-puro resistance	Mammalian selectable marker
Amp resistance	Ampicillin (carbenicillin) bacterial selectable marker
5'LTR	5' long terminal repeat
pUC ori	High copy replication and maintenance of plasmid in *E. coli*
SIN-LTR	3' self inactivating long terminal repeat (Shimada, et al. 1995)
RRE	Rev response element
Zeo resistance	Bacterial selectable marker

Vector Map

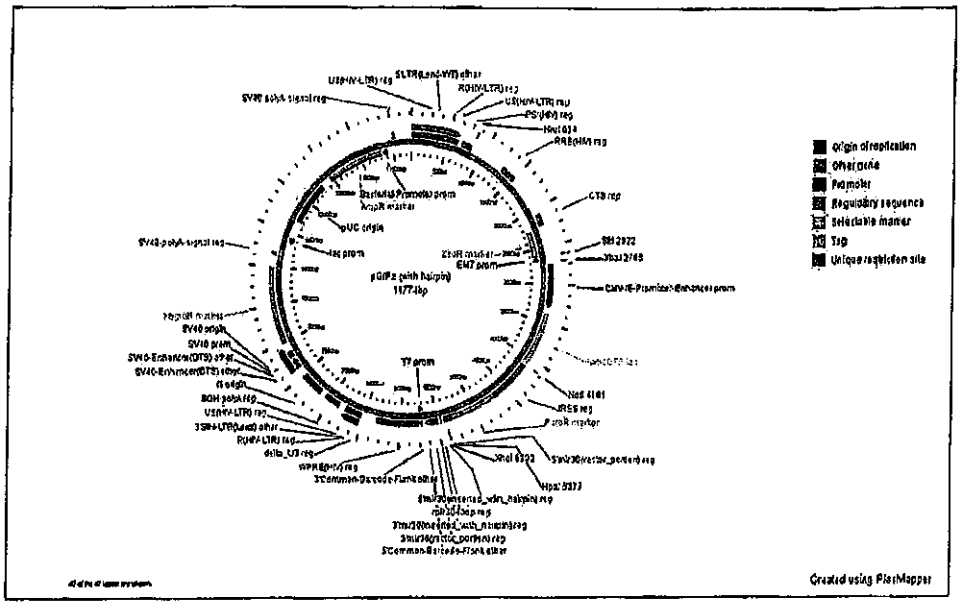


Figure 3. Detailed vector map of pGIPZ lentiviral vector.

Antibiotic Resistance

pGIPZ contains 3 antibiotic resistance markers (Table 2).

Table 2. Antibiotic resistances conveyed by pSM2

Antibiotic	Concentration	Utility
Ampicillin (carbenicillin)	100 µg/ml	Bacterial selection marker (outside LTRs)
Zeocin	25µg/ml	Bacterial selection marker (inside LTRs)
Puromycin	Variable	Mammalian selectable marker

Quality Control

The GIPZ Lentiviral shRNAmir Library has passed through internal QC processes to ensure high quality and low recombination (Figures 4 and 5).

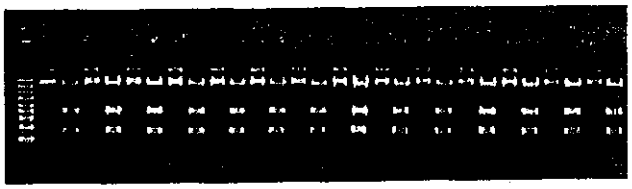


Figure 4. Representative shRNAmir containing pGIPZ lentiviral clones grown for 16 hours at 30°C and the plasmid isolated and normalized to a standard concentration. Clones were then digested with *Sac*II and run out on a gel. The expected band sizes are **1259 bp, 2502 bp, 7927 bp.** No recombinant products are visible. 10 kb molecular weight ladder (10 kb, 7 kb, 5 kb, 4 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb)

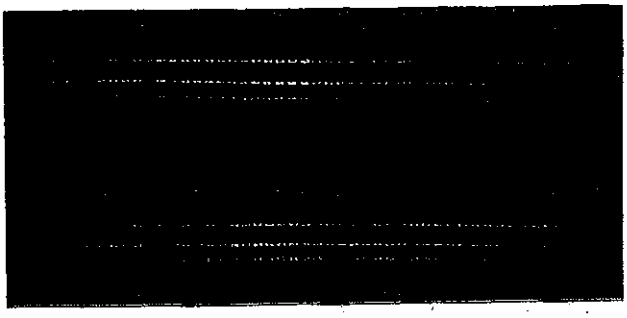


Figure 5. Gel image of a single plate from the GIPZ library cultured for 10 successive generations in an attempt to determine the tendency of the pGIPZ vector to recombine. Each generation was thawed, replicated and incubated overnight for 16 hours at 30°C then frozen, thawed and replicated. This process was repeated for 10 growth cycles. After the 10th growth cycle, plasmid was isolated and normalized to a standard concentration. Clones were then digested with SacII and run on a gel. Expected band sizes **1259 bp, 2502 bp, 7927 bp.** 10 kb molecular weight ladder (10 kb, 7 kb, 5 kb, 4 kb, 3 kb, 2.5 kb, 2 kb, 1.5 kb, 1 kb). The pGIPZ vector appears stable without showing any recombination.

Additional Safety Information

Historically, the greatest safety risk associated with a lentiviral delivery platform stems from the potential generation of recombinant viruses that are capable of autonomous replication. The GIPZ shRNAmir lentiviral platform minimizes these hazards to the greatest degree by combining a disabled viral genome with the proprietary Trans-Lentiviral packaging process. Starting with the HXB2 clone of HIV-1 (GenBank Accession Number K03455), the lentiviral backbone has been modified to eliminate all but the most essential genetic elements necessary for packaging and integration (e.g., 5' LTR, Psi sequences, polypurine tracts, Rev responsive elements and 3' LTR). The resultant self-inactivating (SIN) vector greatly reduces the probability of producing recombinant particles and limits cellular toxicity often associated with expression of HIV genes.

Additional safety features are incorporated by the manufacturing process itself. Generation of GIPZ shRNAmir lentiviral particles requires a packaging step during which the expression construct containing the silencing sequence is enclosed in a viral capsid. Gene functions that facilitate this process (e.g., encoded by the structural genes gag, pol, env, etc.) are distributed amongst multiple helper plasmids which do not contain significant regions of homology. This tactic further minimizes the probability of recombination events that might otherwise generate viruses capable of autonomous replication. Among commercially available lentiviral vector systems, the Trans-Lentiviral Packaging System offers a superior safety profile as the packaging components are separated onto five plasmids. Additionally, expression of gag-pro and tat-rev are under the control of the conditional tetracycline-responsive promoter element (TRE), limiting expression of these viral components strictly to the packaging cell line. A detailed description of the Trans-Lentiviral Packaging System can be found in Wu, et. al. 2000.

With these safety measures in place, GIPZ shRNAmir lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities and should be treated with the same level of caution as any other potentially infectious agent. **Any investigator who purchases Thermo Scientific viral vector products is responsible for consulting with their institution's health and biosafety group for specific guidelines on the handling of lentiviral vector particles. Further, each investigator is fully responsible for obtaining the required permissions for the acceptance of lentiviral particles into their local geography and institution.**

In the US, download the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health, **Biosafety in Microbiological and Biomedical Laboratories (BMBL)**, Fifth Edition, Feb 2007 here: http://www.cdc.gov/biosafety/publications/index.htm.

See also: **NIH Guidelines For Research Involving Recombinant DNA Molecules (NIH Guidelines)**, September 2009, downloadable here: http://oba.od.nih.gov/rdna/nih_guidelines_oba.html

For Biosafety Considerations for Research with Lentiviral Vectors, see http://oba.od.nih.gov/rdna_rac/rac_guidance_lentivirus.html

Protocol I - Replication

Table 3. Materials for plate replication

Item	Vendor	Catalog #
LB-Lennox Broth (low salt)	VWR	EM1.00547.0500
Peptone, granulated, 2 kg - Difco	VWR	90000-368
Yeast Extract, 500 g, granulated	VWR	EM1.03753.0500
NaCl	Sigma	S-3014
Glycerol	VWR	EM-2200 or 80030-956
Carbenicillin or ampicillin	Novagen	69101-3
Zeocin	Invivogen	ant-zn-5p
Puromycin	Cellgro	61-385-RA
96-well microplates	Nunc	260860
Aluminum seals	Nunc	276014
Disposable replicators	Genetix	X5054
Disposable replicators	Scinomix	SCI-5010-OS

For archive replication, grow all pGIPZ clones at 30°C in LB-Lennox (low salt) media plus 25 µg/ml zeocin and 100 µg/ml carbenicillin in order to provide maximum stability of the clones. Prepare media with 8% glycerol* and the appropriate antibiotics.

Replication of Plates

Prepare target plates by dispensing ~160 µl of LB-Lennox (low salt) media supplemented with 8% glycerol* and appropriate antibiotic (25 µg/ml zeocin and 100 µg/ml carbenicillin).

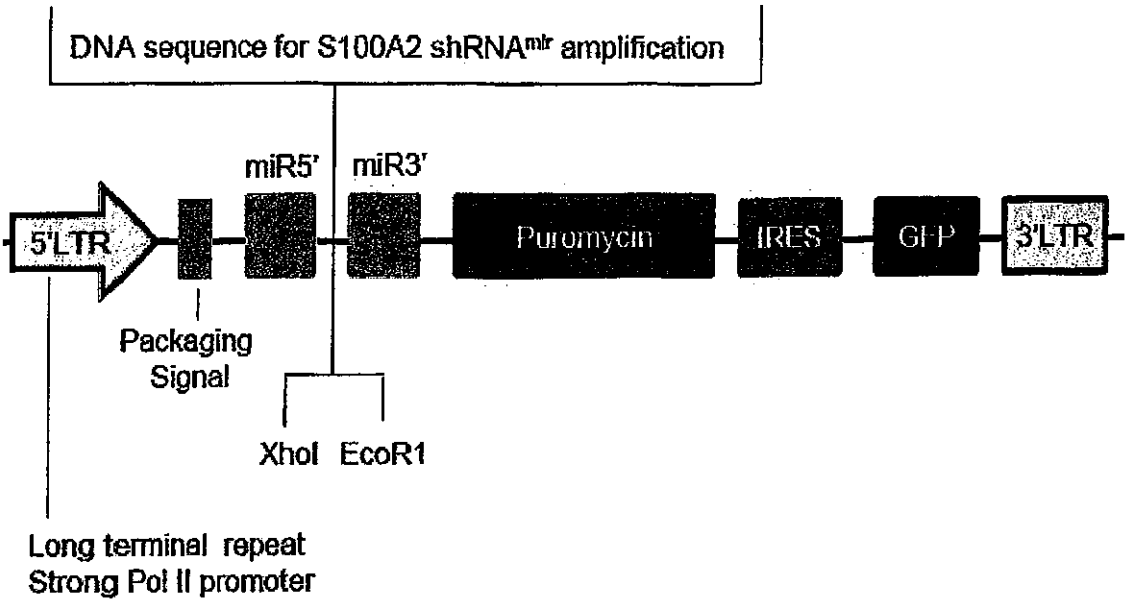


Figure: Plasmid map for S100A2 shRNA vector. The plasmid was obtained as a kind gift from Dr Michael Golding and the map has been adapted from the one provided by him. This is a miR-based shRNA expression cassette and is driven by an RNA polymerase II (Pol II) promoter. The DNA sequence for S100A2 shRNA$_{mir}$ amplification is cloned in between XhoI and EcoR1 sites. The plasmid contains a Puromycin resistance gene to enable selection of stable transfectants. The plasmid also contains an internal ribosome entry site (IRES) for translational initiation in the middle of the mRNA sequence and a green florescent protein (GFP) tag.

Section 4.3

MSDS

Duplicate MSDS'? (Section 1.2)

ThermoFisher SCIENTIFIC

Material Safety Data Sheet

Creation Date 18-Sep-2009 Revision Date 24-May-2010 Revision Number 2

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name Viral Particles - including GIPZ, Lenti-ORF, and shMIMIC

Cat No. VGHXXXX, VGMXXXX, VGRXXXX, RHS4348, RHS4372, RHS4351, HMRXXXX, VSHXXXX (Excluding Arrayed Libraries), OHSXXXX

Synonyms No information available.

Recommended Use For research use only

Company
Thermo Fisher Scientific
Open Biosystem Products
601 Genome Way # 2100
Huntsville, AL 35806 United States
Tel: (303) 604-9499
Fax:(303) 604-9680

Emergency Telephone Number
Chemtrec US: (800) 424-9300
Chemtrec EU: (202) 483-7616

2. HAZARDS IDENTIFICATION

WARNING!

Emergency Overview

Potential Biohazard. Handle in accordance with good industrial hygiene and safety practice. May cause eye, skin, and respiratory tract irritation . Shipped on dry ice.

Appearance Yellow **Physical State** Liquid **odor** No information available

Target Organs None known.

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	May cause irritation
Skin	May cause irritation
Inhalation	May cause irritation of respiratory tract
Ingestion	Ingestion may cause gastrointestinal irritation, nausea, vomiting and diarrhea

Chronic Effects None known.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
DMEM	NA	1 - 99
Viral Particles	NA	1 - 99

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire..

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
None known.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear

NFPA	Health 1	Flammability 0	Instability 0	Physical hazards N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions	Use personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing.
Environmental Precautions	Should not be released into the environment.
Methods for Containment and Clean Up	Soak up with inert absorbent material. Keep in suitable and closed containers for disposal.

7. HANDLING AND STORAGE

Handling	Handle in accordance with good industrial hygiene and safety practice. Wear personal protective equipment. Ensure adequate ventilation. Avoid contact with skin, eyes and clothing. Avoid ingestion and inhalation. This material should be handled at the biosafety level 2 (BSL2) as required by OSHA Bloodborne Pathogen Rule (29 CFR 1910.1030.7).
Storage	Keep container tightly closed. Keep at -80°C.

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Engineering Measures	Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.
Exposure Guidelines	This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

NIOSH IDLH: Immediately Dangerous to Life or Health

Personal Protective Equipment	
Eye/face Protection	Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166
Skin and body protection	Wear appropriate protective gloves and clothing to prevent skin exposure
Respiratory Protection	Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Yellow
odor	No information available
Odor Threshold	No information available.
pH	Not applicable
Vapor Pressure	No information available.
Vapor Density	No information available.

9. PHYSICAL AND CHEMICAL PROPERTIES

Viscosity	No information available.
Boiling Point/Range	Not applicable
Melting Point/Range	No information available.
Decomposition temperature	No information available.
Flash Point	Not applicable
Evaporation Rate	No information available.
Specific Gravity	No information available.
Solubility	No information available.
log Pow	No data available

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Conditions to Avoid	Excess heat.
Incompatible Materials	None known
Hazardous Decomposition Products	None known
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions .	None under normal processing.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information No acute toxicity information is available for this product

Component Information

Irritation No information available.

Toxicologically Synergistic Products No information available.

Chronic Toxicity

Carcinogenicity There are no known carcinogenic chemicals in this product

Sensitization No information available.

Mutagenic Effects No information available.

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity	No information available.
Other Adverse Effects	The toxicological properties have not been fully investigated..
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Do not empty into drains.

Persistence and Degradability	No information available
Bioaccumulation/ Accumulation	No information available
Mobility	No information available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods	Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

TDG

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IATA

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

IMDG/IMO

14. TRANSPORT INFORMATION

UN-No	UN1845
Proper Shipping Name	CARBON DIOXIDE, SOLID
Hazard Class	9
Packing Group	III

15. REGULATORY INFORMATION

International Inventories

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
Not Applicable

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know
Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

WHMIS Hazard Class
D3 Biohazardous infectious materials

16. OTHER INFORMATION

Prepared By	Regulatory Affairs Thermo Fisher Scientific Tel: (412) 490-8929
Creation Date	18-Sep-2009
Print Date	24-May-2010
Revision Summary	"****", and red text indicates revision

Disclaimer

The information provided on this Safety Data Sheet is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS