THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: June 26, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Dr. Tony Rupar
SIGNATURE	
DEPARTMENT	Biochemistry
ADDRESS	Biochemical Genetics Lab, Leonard Bldg Rm 2-9, CPRI, 600 Sanatorium Rd., N6H 3W7
PHONE NUMBER	519-858-2774 X2204 or X2211
EMERGENCY PHONE NUMBER(S)	519-646-9391 (pager)
EMAIL	trupar@uwo.ca

Location of experimental work to be carried out: Building(s) Leonard Bldg, CPRI Room(s) 2-13, 2-25

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: Bethany's Hope Foundation
GRANT TITLE(S): Animal Protocol Title: LV vector-mediated Transfer of Arylsulfatase A and SUMF1 cDNA into a Mouse Model of Metachromatic Leukodystrophy.
Other lab studies have no title as there is no grant.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Dr. Jiahui (James) Liu
Kathie Baer
Cathy Regan

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ⊙YES ○NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)?
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ○YES ⊙NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Cloning strains of E. coli bacteria (JM109, Stbl3, Mach1)	○Yes ⊙No	○Yes ⊙No	○Yes ⊙No	2L	Promega, Invitrogen	⊙1 ○2 ○3
Replication-deficient, self-inactivating lentiviral vectors	○Yes ⊙No	○Yes ⊙No	○Yes ⊙No	0.5L	Made in lab from Invitrogen components	○1 ⊙2 ○3
	○Yes ○No	○Yes ○No	○Yes ○No			○1 ○2 ○3
	○Yes ○No	○Yes ○No	○Yes ○No			○1 ○2 ○3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ⊙YES ○NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	⊙Yes ○No	Specimens from clinical lab	Not applicable
Rodent	⊙Yes ○No	Our mouse colony (C57Bl6 background)	2007-044
Non-human primate	○Yes ⊙No		
Other (specify):	○Yes ⊙No		

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	⦿Yes ○No	HeLa, 293FT	293FT- Invitrogen Corp. HeLa - from another local research lab.
Rodent	⦿Yes ○No	CHO(DG44)	Dr. L. Chasin, Columbia University
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 ○3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ⦿YES ○NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Clinical laboratory	○Yes ⦿No		○1 ⦿2 ○3
Human Blood (fraction) or other Body Fluid	Clinical laboratory	○Yes ⦿No		○1 ⦿2 ○3
Human Organs or Tissues (unpreserved)	Clinical laboratory	○Yes ⦿No		○1 ⦿2 ○3
Human Organs or Tissues (preserved)	Clinical laboratory	○Yes ⦿No		○1 ⦿2 ○3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ⦿YES ○NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ⦿YES, complete table below ○NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid(s)	Gene(s) Transfected	Describe the change that results
JM109 (Promega), Stbl3 (Invitrogen), Mach1 (Invitrogen)	pGem-T-Easy, pEGFP-N1, pcDNA 6.2 DEST, plenti6.3-v5-DEST	Promega, Clontech, Invitrogen	Arylsulfatase A, SUMFI, GFP, lacZ	Generates expression plasmids for these genes to be used in mammalian cells.

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
Replication-deficient, self-inactivating lentiviral (LV)-derived	plenti6.3-V5-DEST, pLP1, pLP2, pLP/VSVG	Invitrogen	Arylsulfatase A, SUMFI, GFP, lacZ	To produce LV-derived vectors to express above genes in transduced mammalian cells

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV ⊙YES, please specify gag, pol, rev ○NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ○YES, specify _____ ⊙NO
- SV 40 Large T antigen ⊙YES ⊙NO
- E1A oncogene ○YES ⊙NO
- Known oncogenes ○YES, please specify _____ ⊙NO
- Other human or animal pathogen and or their toxins ○YES, please specify _____ ⊙NO

4.5 Will virus be replication defective? ⊙YES ○NO

4.6 Will virus be infectious to humans or animals? ⊙YES ○NO

4.7 Will this be expected to increase the containment level required? ○YES ⊙NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? ○YES ⊙NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

5.2 Will the biological agent be able to replicate in the host? ○YES ○NO

5.3 How will the biological agent be administered? _____

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

5.5 Has human ethics approval been obtained? ○YES, number: _____ ○NO ○PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ⊙YES ○NO If no, please proceed to section 7.0

6.2 Name of animal species to be used Mus musculus, C57Bl6, wild-type and ASA knockout

6.3 AUS protocol # 2007-044 (*** No animal experiments done at CPRI site)

6.4 Will any of the agents listed be used in live animals ⊙YES, specify: LV-derived vectors ○NO

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

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

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

8.0 Biological Toxins

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

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

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? ○YES ⊙NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? ○ Initiate and maintain colony, give location: _____
○ "One-time" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? ○YES ◉NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? ○Grow and maintain a crop ○"One-time" use

10.6 Do you do any modifications to the plant? ○YES ○NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? ○YES ○NO
If NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ◉YES, please give country of origin USA
If no, please proceed to Section 12.0 ○NO

11.2 Has an Import Permit been obtained from HC for human pathogens? ○YES ◉NO

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

11.4 Has the import permit been sent to OHS? ○YES, please provide permit # BIO-CPRI-0001 ◉NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

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

SIGNATURE C. A. Lyon

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
◉YES, permit # if on-campus BIO-CPRI-0001
○NO, please certify
○NOT REQUIRED for Level 1 containment

Level 2 certification done on April 1, 2010 by Julie Ryder.

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE C. A. Lyon Date: Feb 24/10

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: Julie Ryder
Date: APRIL 1, 2010

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

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

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

Page 1 of 2
new info
June 3/10

------- Original Message --------
Subject: Re: Biohazardous Agents Registry Form - Rupar lab in CPRI
Date: Thu, 03 Jun 2010 16:37:07 -0400
From: Gail Ryder <Gail.Ryder@LawsonResearch.Com>
To: Jennifer Stanley <jstanle2@uwo.ca>

Jennifer,

Dr. Rupar and I met yesterday. Right now his labs do not meet Level 2+ standards but Dr. Rupar does not feel his research requires this level of biosafety containment. He will discuss this at the upcoming Biosafety meeting this month, if he has not contacted you sooner.

I am on vacation until June 14th and will reply to any other question you may have when I return. Otherwise, I will see you on June 22 at the biosafety meeting.

Thanks,
Gail

Gail Ryder, CRSP
Research Safety Officer

Lawson Health Research Institute
South Street Hospital
375 South Street, Room A210, NR
London, Ontario, Canada N6A 4G5
Tel: (519) 685-8500 x75109
Fax: (519) 432-7367
Pager: x18059
E-mail: Gail.Ryder@LawsonResearch.com
Website: www.lawsonresearch.com

>>> Jennifer Stanley <jstanle2@uwo.ca> 2010/06/02 04:01 PM >>>
Dr. Rupar & Gail -

Please let me know the status of this - the Committee is meeting next week and would like an update.

Please note, Dr. Rupar, that a project summary is required for the in vitro work you do at CPRI. Please forward it to me asap.

Regards
Jennifer

-------- Original Message --------
Subject: Re: Fwd: Rupar lab in CPRI
Date: Mon, 17 May 2010 11:20:25 -0400
From: Gail Ryder <Gail.Ryder@LawsonResearch.Com>
To: jstanle2@uwo.ca, trupar@uwo.ca

Ok I will work on this with Dr. Rupar and be in touch with you Jennifer.

Gail Ryder, CRSP
Research Safety Officer

The Lawson Health Research Institute
South Street Hospital
375 South Street, Room A210, NR
London, Ontario, Canada N6A 4G5
Tel: (519) 685-8500 x75109
Fax: (519) 432-7367
Pager: x18059
E-mail: gail.ryder@lhsc.on.ca
Website: www.lhrionhealth.ca
>>> Jennifer Stanley<jstanle2@uwo.ca> 05/17/10 11:11 AM>>>

Hello Dr. Rupar and Gail -

Per the Biohazards Subcommittee meeting Friday, May 14th, this project is Level 2 plus.

Please let me know when this inspection is complete so that the project can be approved.

Thanks!
Jennifer

Subject: Rupar lab in CPRI
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Mon, 19 Apr 2010 14:30:52 -0400
To: rsn@uwo.ca

Hi Gail

My understanding is that Public Health Agency of Canada requires Level 2 plus for this research (unless there has been a change in the research done since 2007) - please see the attached information. The Public Health Agency of Canada checklist for the Level 2 plus labs is also attached.

The in vivo work done on campus is done with Level 2 plus Level 3 precautions.

Regards,
Jennifer

On 4/9/2010 2:48 PM, Gail Ryder wrote:

> Hi Jennifer,
>
> Dr. Rupar's lab is off site at the Child and Parent Resource Institute. I just certified his lab Level 2 PHAC on April 1st, 2010 in order that we can process his BARF. I also am in the process of registering his building with HPTA. At this point his laboratory is not officially a Level 2+ lab due to the fact that Dr. Rupar stated his lentiviral/HIV work is replication-deficient and self-inactivating and in section 4.7 of the form checked NO for whether the containment level needed to increase.
>
> LHSC only has one official level 2+ approved room for Lentiviral work and that is at the VRL at Victoria Hospital. If we need to certify Dr. Rupar's lab Level 2+ let me know and I'll see if it's possible. However, currently it meets all the Level 2 guidelines and in addition he uses a certified BSC, dedicated incubators, proper disinfectants, his waste is autoclaved, they have disposable lab coats, the lab doors where he conducts his research and stores his pathogens are closed and lockable, lab coats are hung inside the lab near the exit, mice brought into the lab remain in the lab, centrifuge in the lab is sealed or opened in the BSC, etc. Since we don't have an official checklist that we can use for Level 2+ like we do for Level 2 we have look at each situation on a case by case basis. Please let me know what concerns you have in mind that I may not have mentioned in this email and we can address it from there.
>
> Thanks,
> Gail
>
> Gail Ryder, CRSP

Research Safety Officer

Lawson Health Research Institute
South Street Hospital
375 South Street, Room A210, NR
London, Ontario, Canada N6A 4G5
Tel: (519) 685-8500 x75109
Fax: (519) 432-7367
Pager: x18059
E-mail: Gail.Ryder@LawsonResearch.com
Website: www.lawsonresearch.com

Jennifer Stanley <jstanle2@uwo.ca> 2010/04/08 04:22 PM >>>

Hi Gail

Is Dr. Rupar's lab containment Level 2 plus?

Regards
Jennifer

cl2+ additional items checklist.doc	**Content-Type:** application/msword **Content-Encoding:** base64

Rupar_Level_2_plus_2007_information.pdf

Rupar_Level_2_plus_2007_information.pdf	**Content-Type:** application/pdf **Content-Encoding:** base64

Ontario

Ministry of Children and Youth Services | Ministère des Services à l'enfance et à la jeunesse

FASCIMILIE ROUTING SLIP
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South West Region | **district sud-ouest**

CPRI
600 Sanatorium Road
London ON N6H 3W7

CPRI
600 chemin Sanatorium
London (Ontario) N6H 3W7

Date: **April 13, 2007**

Time/Hours/Heure:

To/Destinataire: **OHS**

Name/Nom: **Jennifer Stanley**

Location/Ville: **SLB 295**

Telephone/Téléphone: **519-661-2111 X81135**

From/Expédite **CPRI-Biochemical Genetics Laboratory**

Name/Nom: **Cathy Regan**

Location/Ville: **London**

Telephone/Téléphone: **(519) 858-2774, Ext. 2211**

Facsimile number/ N° de télécopieur	Speed dial number/ N° abrégé	Facsimile number/ N° de télécopieur	Number of pages/ Nombre de suivre
519-661-3420		**(519) 858-1063**	**3**

Subject/Sujet:
From Dr. Tony Rupar

Additional instruction/Directives additionnelles:

☐ **Valid consent on file**

☒ **Not applicable**

Verified by: _____

Please find attached the information Tony referred to in his e-mail.

Sent by/Envoyé par

Date

file: Rupar

Public Health Agency of Canada / Agence de santé publique du Canada

Your file / Votre référence

Our file / Notre référence

Required Level 3 operational procedures for RG2+ organisms are:

- There must be a program for the management of biological safety issues in place with appropriate authority to oversee safety and containment practices.
- Everyone entering the containment laboratory must have completed a training course in procedures specific to the containment laboratory and must show evidence of having understood the training; training must be documented and signed by the employee and supervisor.
- A protocol specific to the operation of the laboratory must be developed and read by personnel; employees must certify in writing that they have understood the material in the protocol. This should include entry and exit protocols for people, animals, equipment, samples and waste. General protocols must be supplemented with protocols specific to each project in progress.
- Personnel must have demonstrated proficiency in microbiological practices and techniques.
- Routine laboratory cleaning must be done by personnel using the containment facility or by specific personnel dedicated and trained for this task.
- The containment laboratory must be kept locked when it is unoccupied.
- Infectious agents should be stored inside the containment laboratory; agents stored outside of the zone must be kept locked, in leakproof containers; emergency response procedures are to take into account the existence of such infectious agents outside of the containment laboratory.
- Personal items such as purses and outdoor clothing must not be brought into the containment laboratory.
- Drainage traps must be filled with liquid (i.e., through regular sink usage, automatic primers or by filling traps in areas that are not frequently used).
- Personnel entering the containment laboratory must remove street clothing and jewelry, and change into dedicated laboratory clothing and shoes; dedicated laboratory clothing and shoes must be removed before leaving the containment laboratory in a manner that

minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing. The use of full coverage protective clothing (i.e., completely covering all street clothing) is an acceptable alternative. When a known or suspected exposure may have occurred, all clothing, including street clothing, requires appropriate decontamination.

- An additional layer of protective clothing (i.e., solid-front gowns with tight-fitting wrists, gloves, respiratory protection(7)) may be worn over laboratory clothing when infectious materials are directly handled and should be removed after completion of work (e.g., dedicated for use at the BSC).

- Centrifugation of infectious materials must be carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC.

- Animals or arthropods that have been experimentally infected must remain in the laboratory or appropriate animal containment facility.

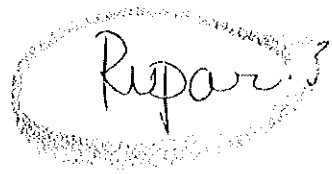
- When a known or suspected aerosol exposure may have occurred, protocols based on a local risk assessment must be in place to determine whether showering is required on exit from the laboratory. If no shower is available inside the containment laboratory, a procedure must be in place to replace a body shower before exiting the laboratory in the event of a spill.

- All activities with infectious materials are conducted in a BSC; if this is not possible, other primary containment devices in combination with personal protective clothing and equipment must be used; no work with open vessels containing infectious materials is conducted on the open bench.

- Emergency procedures for failure of air handling systems and other containment emergencies must be written, easily accessible and followed.

- In the event of life-threatening emergencies, personal health and safety are a priority; exit protocols must be established whereby routine procedures might be bypassed; a reporting area must be identified where further steps must be taken (e.g., disinfecting footwear, changing, showering).

Note that the general practices and the level 2 operational procedures must also be followed in additions to the requirements above. Please refer to Chapter 3 of the Laboratory Biosafety Guidelines 3rd Edition for these requirements.
http://www.phac-aspc.gc.ca/ols-bsl/lbg-ldmbl/index.html



-------- Original Message --------
Subject: Re: Lentiviral animal work & containment required
Date: Mon, 08 Jan 2007 10:30:53 -0500
From: Lise Murphy <lise_murphy@hc-sc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

The Public Health Agency of Canada permit that you have for this vector system does in fact indicate that you work at level 2 containment with level 3 operational practices. It will also states if you can work in vivo with those vector systems.

I hope that helps,

Lise

Lise Murphy, MSc.
A/ Biosafety Specialist/ Spécialiste en biosécurité
Office of Laboratory Security/Bureau de la sécurité des laboratoires
Public Health Agency of Canada/ Agence de santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A Ottawa, Ontario, Canada K1A 0K9
Tel: (613) 957-1779
Fax: (613)941-0596

Repar

-------- Original Message --------
Subject: Re: Containment Level?
Date: Fri, 13 Jul 2007 09:44:20 -0400
From: Lise Murphy <lise_murphy@hc-sc.gc.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Currently we are recommending Containment Level 2 physical with Containment Level 3 operational practices and an emphasis on strictly limiting the use of needles and sharps for both in vitro and in vivo protocols. A local risk assessment of the in vivo work should be done to determine at which point the CL3 operational practices are no longer warranted (ie: shedding data, wound healing, viral tropism, is the virus replication deficient, which generation of lentiviral vector is it?).

Hope that this helps,

Lise

Lise Murphy, MSc.
Regulatory Technologist/ technologiste en réglementation
Office of Laboratory Security/Bureau de la sécurité des laboratoires
Public Health Agency of Canada/ Agence de santé publique du Canada
100 ch. Colonnade Rd. AL: 6201A Ottawa, Ontario, Canada K1A 0K9
Tel: (613) 957-1779
Fax: (613)941-0596

Containment Level 2+ 3 Operational – Additional Items Checklist

Q.4. **(Chapter 3.1.3)**	Is a program in place (with appropriate authority to oversee safety and containment practices) for the management of biological safety issues?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.19. **(Chapter 3.1.3)**	Is a containment check performed before entering the containment laboratory (e.g. verify correct readings on the pressure monitoring device)?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.20. **(Chapter 3.1.3)**	Does personnel remove street clothing and change into dedicated laboratory clothing and shoes when entering the laboratory? Note: the use of full coverage protective clothing is an acceptable alternative. Laboratories manipulating organisms, such as HIV, that are not infectious via inhalation are not required to remove street clothing.	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.24. **(Chapter 3.1.3)**	If an additional layer of protective clothing (e.g. solid-front gowns with tight-fitting wrists, gloves, respiratory protection) is worn over laboratory clothing when handling infectious materials, is it removed after completion of work (e.g. dedicated for use at the BSC)?	RECOMMENDED ☐ Yes ☐ No ☐ N/A
Q.25. **(Chapter 3.1.3)**	Are dedicated laboratory clothing and shoes removed before leaving the laboratory in a manner that minimizes any contamination of the skin with the potentially contaminated dedicated laboratory clothing?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.34. **(Chapter 3.1.3)**	Are infectious agents stored inside the containment laboratory?	RECOMMENDED ☐ Yes ☐ No ☐ N/A
Q.35. **(Chapter 3.1.3)**	If agents are stored outside of the containment laboratory are they kept in leakproof containers in a restricted area? Note: emergency response procedures must take into account the existence of infectious agents that are stored outside of the containment area.	MANDATORY ☐ Yes ☐ No ☐ N/A

Containment Level 2+ 3 Operational – Additional Items Checklist

Q.36. **(Chapter 3.1.3)**	Are all activities with infectious materials conducted in a BSC?	RECOMMENDED ☐ Yes ☐ No ☐ N/A
Q.37. **(Chapter 3.1.3)**	Are other primary containment devices in combination with personal protective clothing and equipment used if it is not possible to conduct all activities with infectious materials in a BSC?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.38. **(Chapter 3.1.3)**	Is the centrifugation of infectious materials carried out in closed containers placed in sealed safety cups or rotors that are unloaded in a BSC?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.129. **(Chapter 3.1.3)**	In the event of an emergency, is an exit protocol in place whereby routine procedures might be bypassed? Note: in the event of life-threatening emergencies, personal health and safety are always a priority.	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.130. **(Chapter 3.1.3)**	Has a reporting area been identified where further steps might be taken (e.g. disinfecting footwear, changing, showering)?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.138. **(Chapter 3.1.3)**	Are the general operational protocols supplemented with protocols specific to each project in progress?	MANDATORY ☐ Yes ☐ No ☐ N/A
Q.140. **(Chapter 3.1.3)**	Have personnel demonstrated proficiency in microbiological practices and techniques?	MANDATORY ☐ Yes ☐ No ☐ N/A

Biosafety Features of the System

Introduction

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

Biosafety Features of the ViraPower™ Lentiviral System

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

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

Continued on next page

Biosafety Features of the System, Continued

Biosafety Level 2

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

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

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

Important

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

MATERIAL SAFETY DATA SHEET

Revised 1/30/03
Replaces (None)
Printed 1/30/03

VIRAPOWER PKG. MIX 195 UG
INVITROGEN CORPORATION
MSDS ID: 442050

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

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

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name:
ViraPower Lentiviral Support Kit

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therpeutic, or other clinical uses.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
TRIZMA BASE	MIXTURE	10 - 30

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if swallowed.
Harmful if absorbed.
Harmful by inhalation.
****

Potential Health Effects:
Eye:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin:
Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage.
Upon prolonged or repeated exposure, harmful if absorbed through the skin.
May cause minor systemic damage.

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.
Harmful! Can cause systemic damage (see "Target Organs).

Ingestion:
Mildly irritating to mouth, throat, and stomach. Can cause abdominal discomfort.
Harmful if swallowed. May cause systemic poisoning.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin:
Wash with soap and water. Remove contaminated clothing and launder. Get medical attention if irritation develops or persists.

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.

4. FIRST AID MEASURES (CONT.)

Ingestion:
Do not induce vomiting and seek medical attention immediately. Drink two glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.
Use water spray/fog for cooling.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Includes carbon dioxide, carbon monoxide, dense smoke.

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider

6. ACCIDENTAL RELEASE MEASURES (CONT.)

the expertise of employees in the area responding to the spill. Prevent the spread of any spill to minimize harm to human health and the environment if safe to do so. Wear complete and proper personal protective equipment following the recommendation of Section VIII at a minimum. Dike with suitable absorbent material like granulated clay. Gather and store in a sealed container pending a waste disposal evaluation.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
Keep closed or covered when not in use.

Storage Procedures:
Store in a cool dry place. Isolate from incompatible materials.
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
TRIZMA BASE	Not established.	Not established.

Engineering Controls:
No exposure limits exist for the constituents of this product. Use local exhaust ventilation or other engineering controls to minimize exposures and maintain operator comfort.

Personal Protective Equipment:

Eye:
An eye wash station must be available where this product is used.
Wear chemical goggles.

Skin:
Wear protective gloves. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work .

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

Gloves should be used as minimum hand protection.

Respiratory:
A respiratory protection program that meets OSHA's 29 CFR 1910.134 and ANSI Z88.2 requirements must be followed whenever workplace conditions warrant a respirator's use.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong alkalies. Strong oxidizing agents.

Hazardous Decomposition Products:
Carbon dioxide. Carbon monoxide. Nitrogen oxides.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
Not determined.

11. TOXICOLOGICAL INFORMATION (CONT.)

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
TRIZMA BASE: 5900 MG/KG

Target Organs: No data found.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Hazard Class:
Subsidiary Hazards:
ID Number:

14. TRANSPORT INFORMATION (CONT.)

Packing Group:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
TRIZMA BASE	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
Not applicable.

PRODUCT CLASSIFICATION:
Not classified as hazardous

Component	EINECS Number
TRIZMA BASE	Not established.

16. OTHER INFORMATION

HMIS Rating 0-4:
FIRE: Not determined.
HEALTH: Not determined.
REACTIVITY: Not determined.

16. OTHER INFORMATION (CONT.)

Abbreviations
N/A - Data is not applicable or not available
SARA - Superfund and Reauthorization Act
HMIS - Hazard Material Information System
WHMIS - Workplace Hazard Materials Information System
NTP - National Toxicology Program
OSHA - Occupational Health and Safety Administration
IARC - International Agency for Research on Cancer
PROP 65 - California Safe Drinking Water and Toxic Enforcement Act of 1986
EINECS - European Inventory of Existing Commercial Chemical Substances

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 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 PARTICULAR PURPOSE.

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Material Safety Data Sheet

Revision Date: 02-Mar-2009

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

Product code 510035
Product name 293FT Cells

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

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	7-13

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

3. HAZARDS IDENTIFICATION

Emergency Overview
Components of the product may be absorbed into the body through the skin

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
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	1
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

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
dimethylsulfoxide	-	-	-	-

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	Impervious butyl rubber gloves. Nitrile gloves are not recommended. Some brands of Nitrile gloves have breakthrough times of five minutes.. Nitrile gloves are not recomended. Some brands of Nitrile gloves have breakthrough times of five minutes.
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	soluble	

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

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	Mild eye irritation.
Skin	moderate skin irritation. Components of the product may be absorbed into the body through the skin.
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

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

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
dimethylsulfoxide	Listed	Listed	Listed	Listed	-	Listed

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

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
dimethylsulfoxide	-	-	-	-	-

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

WHMIS hazard class:
D2B Toxic materials

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

invitrogen™

Material Safety Data Sheet

Revision Date: 12-Feb-2008

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

Product code 500326
Product name STBL 3 One Shot

Company/Undertaking Identification

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

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
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

3. HAZARDS IDENTIFICATION

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

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

3. HAZARDS IDENTIFICATION

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	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	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

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

Project Summary – Rupar Lab

Metachromatic leukodystrophy (MLD) is a progressive neurodegenerative lysosomal storage disease. It is caused by a deficiency of the enzyme arylsulfatase A (ARSA) and is inherited in an autosomal recessive manner. There is no treatment for MLD.

The ARSA -/- mouse is a biochemical and genetic model for MLD. The objective of this project is to develop approaches to treating the mouse model of MLD.

Two *in vivo* experimental approaches are in progress:

1. The intracranial injection of replication deficient lentivirus-derived gene transfer vectors into a lateral ventricle of ARSA -/- mice is underway. Vectors have been created containing transgenes of GFP, ARSA-V5 (V5 is a short epitope tag), and SUMF-1 (encodes an enzyme for an obligatory post-translational modification of ARSA).

2. The intracranial injection of ARSA-V5 produced *in vitro* in Chinese hamster ovary (CHO) cells by transduction with replication deficient lentivirus-derived gene transfer vectors is beginning.

All vectors are prepared by quadruple transfection of the 293FT packaging cells using plasmids provided by Invitrogen in addition to the transgene containing plasmid prepared in our laboratory. The vectors are pseudotyped with the VSV-G protein and use a CMV promoter. Plasmid maps are provided.

Below are listed current agents and use in our laboratory:

Bacterial cultures:
1. JM109 – general plasmid cloning
2. Stbl3 and Mach1 – cloning of larger plasmids or plasmids containing long terminal repeat sequences.

DNA plasmids:
1. pGem-T-easy – general cloning
2. pEGFP-N1 – contains the GFP expression cassette to fluorescently label fusion proteins
3. pcDNA 6.2 DEST – mammalian expression vector
4. pLenti 6.3-V5-DEST – lenti expression vector – genes of interest (ie. ARSA) are cloned into this vector for expression
5. pLP, pLP2, pLP/VSVG – packaging plasmids co-transfected with lenti expression plasmid to produce vector.

Established cell lines:
1. CHO (DG44) – used to produce recombinant ARSA-V5 protein
2. 293FT – lenti vector producer cell line

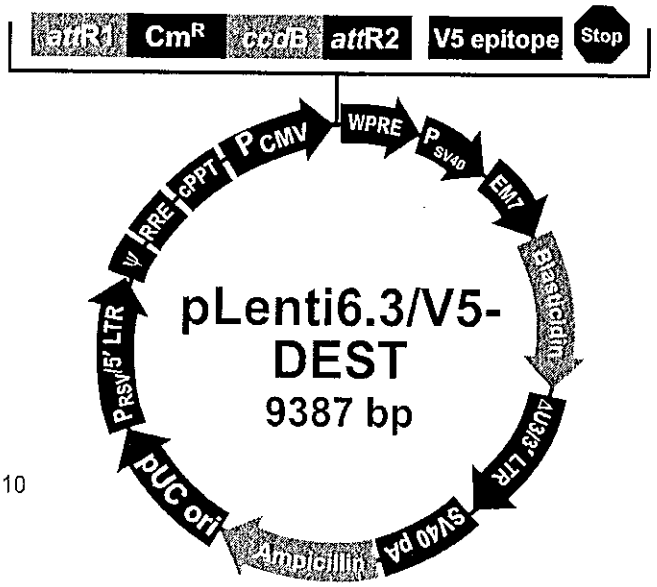
3. HeLa – to titer vector

Primary cell lines:

1. Human fibroblasts – from MLD individuals (and normal controls) to test vectors for expression of ARSA.
2. Mouse brain cultures – from ARSA -/- mice and control mice - to test uptake and expression of vector in cell types that are the target cell types of treatment.

Human tissues:

1. Blood and/or blood fractions – used to establish a blood dot card assay for MLD
2. Fibroblast cells – see above.



Comments for pLenti6.3/V5-DEST
9387 nucleotides

RSV/5′ LTR hybrid promoter: bases 1-410
- RSV promoter: bases 1-229
- HIV-1 5′ LTR: bases 230-410

5′ splice donor: base 520
HIV-1 psi (ψ) packaging signal: bases 521-565
HIV-1 Rev response element (RRE): bases 1075-1308
3′ splice acceptor: base 1656
3′ splice acceptor: base 1684
cPPT: bases 1801-1923
CMV promoter: bases 1935-2519
*att*R1 site: bases 2568-2692
Chloramphenicol resistance gene (Cm^R): bases 2801-3460
*ccd*B gene: bases 3802-4107
*att*R2 site: bases 4148-4272
V5 epitope: bases 4325-4366
WPRE: bases 4385-4982
SV40 promoter: bases 4993-5301
EM7 promoter: bases 5356-5422
Blasticidin resistance gene: bases 5423-5821
ΔU3/3′ LTR: bases 5907-6141
- ΔU3: bases 5907-5960
- 3′ LTR: bases 5961-6141

SV40 polyadenylation signal: bases 6213-6344
bla promoter: bases 7203-7301
Ampicillin (*bla*) resistance gene: bases 7302-8162
pUC origin: bases 8307-8980

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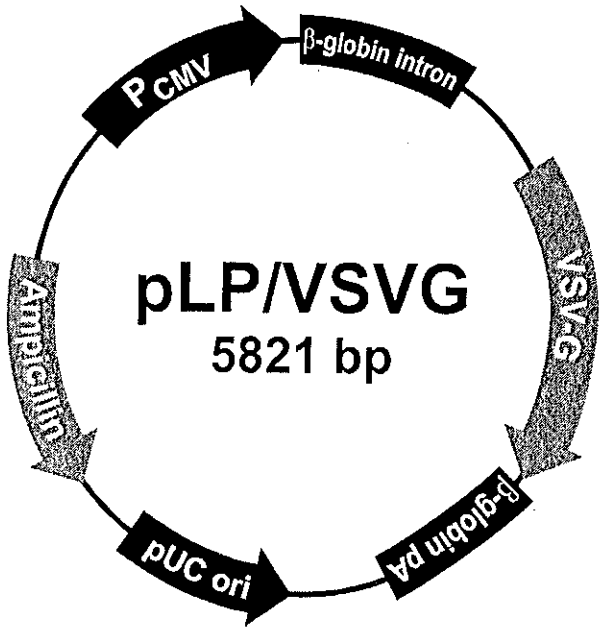

Comments for pLP/VSVG
5821 nucleotides

CMV promoter: bases 1-747
TATA box: bases 648-651
Human β-globin intron: bases 880-1320
VSV G glycoprotein (VSV-G): bases 1346-2881
Human β-globin polyadenylation signal: bases 3004-3769
pUC origin: bases 3927-4600 (C)
Ampicillin (*bla*) resistance gene: bases 4745-5605 (C)
bla promoter: bases 5606-5704 (C)

C=complementary strand

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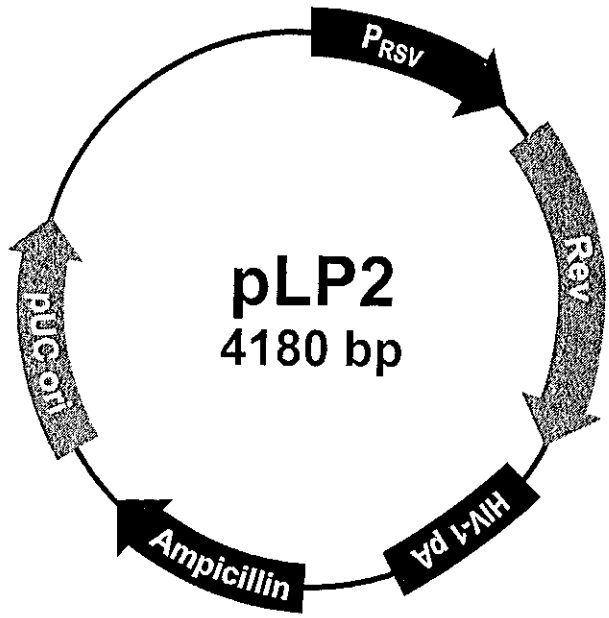

Comments for pLP2
4180 nucleotides

RSV enhancer/promoter: bases 1-271
TATA box: bases 200-207
Transcription initiation site: base 229
RSV UTR: bases 230-271
HIV-1 Rev ORF: bases 391-741
HIV-1 LTR polyadenylation signal: bases 850-971
bla promoter: bases 1916-2014
Ampicillin (*bla*) resistance gene: bases 2015-2875
pUC origin: bases 3020-3693

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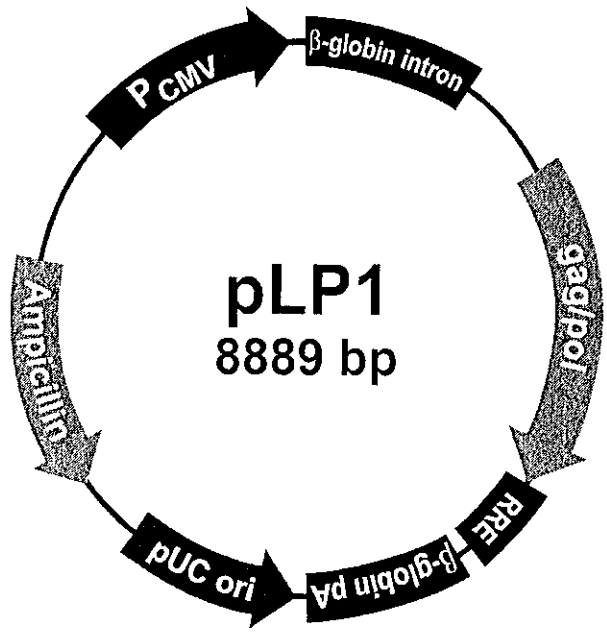

Comments for pLP1
8889 nucleotides

CMV promoter: bases 1-747
  TATA box: bases 648-651
Human β-globin intron: bases 880-1320
HIV-1 gag/pol sequences: bases 1355-5661
  gag coding sequence: bases 1355-2857
  gag/pol frameshift: base 2650
  pol coding sequence: bases 2650-5661
HIV-1 Rev response element (RRE): bases 5686-5919
Human β-globin polyadenylation signal: bases 6072-6837
pUC origin: bases 6995-7668 (C)
Ampicillin (*bla*) resistance gene: bases 7813-8673 (C)
bla promoter: bases 8674-8772 (C)

C=complementary strand

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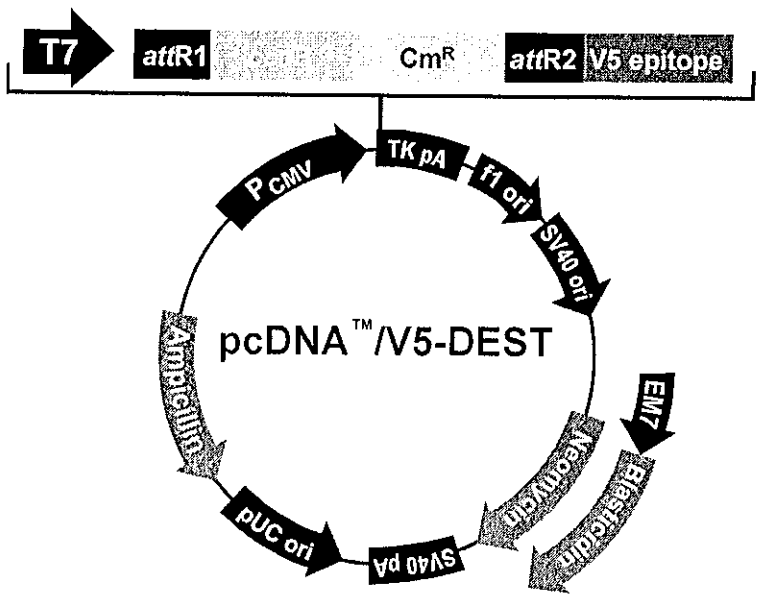

Comments for:	pcDNA™3.2/V5-DEST 7711 nucleotides	pcDNA™6.2/V5-DEST 7341 nucleotides
CMV promoter:	232-819	232-819
T7 promoter/priming site:	863-882	863-882
*att*R1 site:	911-1035	911-1035
*ccd*B gene (c):	1464-1769	1464-1769
Chloramphenicol resistance gene (c):	2111-2770	2111-2770
*att*R2 site:	3051-3175	3051-3175
V5 epitope:	3201-3242	3201-3242
V5 reverse priming site:	3210-3230	3210-3230
TK polyadenylation signal:	3269-3540	3269-3540
f1 origin:	3576-4004	3576-4004
SV40 early promoter and origin:	4031-4339	4031-4339
Neomycin resistance gene:	4414-5208	---
EM7 promoter:	---	4394-4460
Blasticidin resistance gene:	---	4461-4859
SV40 early polyadenylation signal:	5384-5514	5017-5147
pUC origin (c):	5897-6570	5530-6200
Ampicillin (*bla*) resistance gene (c):	6715-7575	6345-7205
bla promoter (c):	7576-7674	7206-7304

(c) = complementary strand

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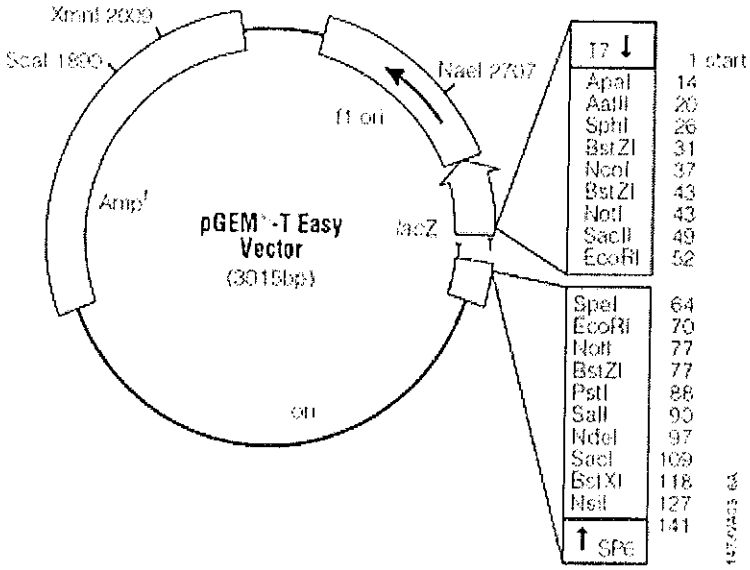
pgem®-t easy vector system i

a1360



Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

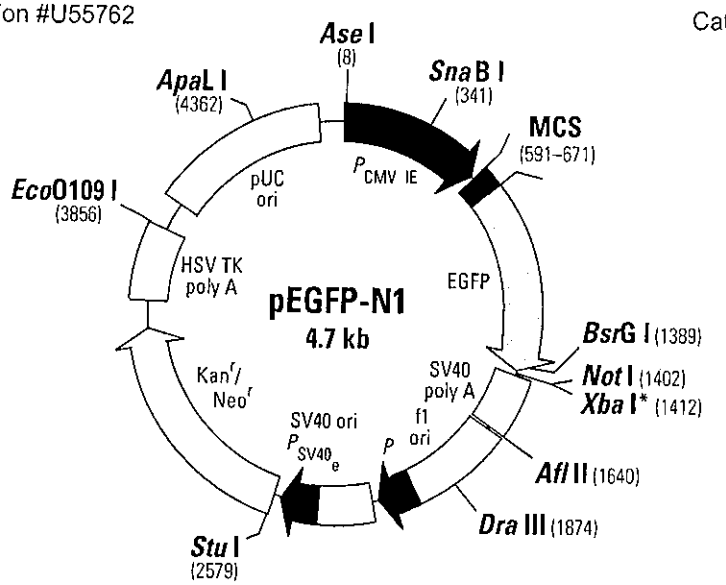
Vector Information

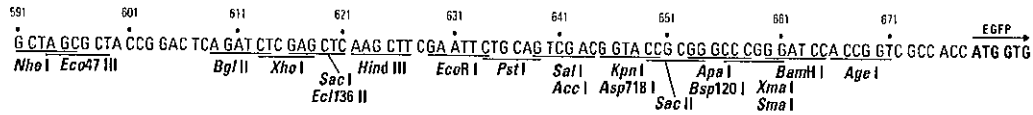
pEGFP-N1 Vector Information

PT3027-5

GenBank Accession #U55762

Catalog #6085-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1 Vector. All restriction sites shown are unique. The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ and make fresh DNA.

Description

pEGFP-N1 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neor), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-N1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

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Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR29972; published 03 October 2002)

Use

Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N1 can also be used simply to express EGFP 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
- MCS: 591–671
- Enhanced green fluorescent protein (EGFP) gene
  - Kozak consensus translation initiation site: 672–682
  - Start codon (ATG): 679–681; Stop codon: 1396–1398
  - Insertion of Val at position 2: 682–684
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 871–876
  - His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1552–1557 & 1581–1586; mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104 (Packages the noncoding strand of EGFP.)
- Bacterial promoterfor expression of Kan^r gene:
  - –35 region: 2166–2171; –10 region: 2189–2194
  - Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
  - 21-bp repeats: 2425–2445, 2446–2466 & 2468–2488
  - Early promoter element: 2501–2507
  - Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences: start codon (ATG): 2629–2631; stop codon: 3421–3423
  - G→A mutation to remove *Pst* I site: 2811
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

Propagation in *E. coli*

- Suitable host strains: DH5a, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II.* Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

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Material Safety Data Sheet

Revision Date: 15-Oct-2007

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

Product code 462053
Product name pLenti6.3/V5-DEST Gateway® Vector

Company/Undertaking Identification

INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
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

3. HAZARDS IDENTIFICATION

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

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

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

3. HAZARDS IDENTIFICATION

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	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	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

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

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
Emergency Phone Number: CHEMTREC 1-800-424-9300
Number for Information: 1-800-356-9526/608-274-4330

ATTENTION: **Safety Officer**

Per OSHA 29CFR1910.1200, Part IV of the Controlled Products Regulations (CPR) of Canada, the Commonwealth of Australia [NOHSC:1005, 1008(1999)] and the latest amendments to the European Union Directive 67/548/EC, the following products do not require a Material Safety Data Sheet (MSDS). This decision is based upon our hazard determination process of evaluating available scientific evidence. The chemical, physical, and toxicological properties of these products may not, as yet, have been thoroughly investigated; therefore, we recommend the use of gloves, lab coats, and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

Last Updated: July 12, 2008

A

A109	Upstream Control Primer
A110	Downstream Control Primer
A111	Positive Control RNA with Carrier
A117	Protein Isoaspartyl Methyltransferase
A118	Reaction 5X Buffer
A119	Isoasp Delta Sleep Inducing Peptide
A120	S-adenosyl-L-Methionine
A1250	Access RT-PCR System
A1260	Access RT-PCR Introductory System
A1280	Access RT-PCR System, 500 Reactions
A1281	Access RT-PCR Core Reagents
A129	Wizard® SV Minicolumns
A130	Collection Tubes
A131	Wizard® Plus SV Column Wash Solution
A1311	Wizard® SV Wash Solution
A1318	Wizard® SV 96 Wash Solution, 370ml
A133	Vacuum Adapter
A1331	Vacuum Adapter, 20/pk
A1360	pGEM®-T Easy Vector System I
A137	pGEM® T-Easy
A1380	pGEM®-T Easy Vector System II

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

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

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).

SECTION I
Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
Physical data

Pink or red aqueous liquid

SECTION III
Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV
Fire and explosion

Not applicable

SECTION V
Reactivity data

Stable. Hazardous polymerization will not occur.

SECTION VI
Method of disposal

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.
Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

SECTION VII
Special protection information

For Biosafety Level 1 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

For Biosafety Level 2 Cell Lines

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION VIII
Special precautions or comments

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.

February 2002

invitrogen™

Material Safety Data Sheet

Revision Date: 20-Jun-2005

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

Product code 500324
Product name MACH 1 ONE SHOT COMP CELL

Contact manufacturer
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1600 FARADAY AVENUE
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GIBCO PRODUCTS
INVITROGEN CORPORATION
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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

3. HAZARDS IDENTIFICATION

Emergency Overview
No information available

Form
Solid

Principle Routes of Exposure/ Potential Health effects

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

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
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	No information available.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	No information available

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	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	No information available.
Bioaccumulation	No information available.

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
Not regulated

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product contains the following HAPs:

U.S. State Regulations

California Proposition 65
This product contains the following Proposition 65 chemicals:

WHMIS hazard class:
Not determined

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

Promega Corporation
2800 Woods Hollow Road
Madison, WI 53711
Emergency Phone Number: CHEMTREC 1-800-424-9300
Number for Information: 1-800-356-9526/608-274-4330

ATTENTION: **Safety Officer**

Per OSHA 29CFR1910.1200, Part IV of the Controlled Products Regulations (CPR) of Canada, the Commonwealth of Australia [NOHSC:1005, 1008(1999)] and the latest amendments to the European Union Directive 67/548/EC, the following products do not require a Material Safety Data Sheet (MSDS). This decision is based upon our hazard determination process of evaluating available scientific evidence. The chemical, physical, and toxicological properties of these products may not, as yet, have been thoroughly investigated; therefore, we recommend the use of gloves, lab coats, and eye protection when working with these or any chemical reagents. Promega assumes no liability for damage resulting from handling or contact with these products.

Last Updated: July 12, 2008

H-I-J-K-L

H5001	Boric Acid
H5003	Boric Acid
H507	Glycine
H5071	Glycine
H5073	Glycine
H5151	Tween® 20, 500ml
H5152	Tween® 20, 100ml
H5271	Sodium Chloride, 500gm
H5273	Sodium Chloride, 1kg
H5302	HEPES (free acid), 100gm
H5303	HEPES (free acid), 500gm
H5433	Glycerol, 1000ml
HSMC	GoTaq® Hot Start Colorless Master Mix
HSMG	GoTaq® Hot Start Green Master Mix
HSFLX	GoTaq® Hot Start Polymerase, Sample
K315	Packagene® System
K3152	Packagene® Lambda DNA Packaging System, 11 Ext
K3154	Packagene® Lambda DNA Packaging System, 6 Ext
K4000	DNA Quantitation System
K401	DNA Quantitation Buffer Solution
K402	NDPK from Bakers Yeast
K403	DNA Quantitation DNA Standard
K997	Packagene® Control DNA
K998	LE392 Bacterial Strain

K9981	Bacterial Strain LE392
L100	JM109 Competent Cells
L1001	JM109 Competent Cells, Subcloning Efficiency, 1ml
L101	HB101 Competent Cells
L1011	HB101 Competent Cells, Subcloning Efficiency, 1ml
L1020	E. coli S30 Extract System for Circular DNA
L1030	E. coli S30 Extract System for Linear Templates
L105	Luciferase ICE T7 Control DNA
L106	pF25A ICE T7 Flexi® Vector
L1061	pF25A ICE T7 Flexi® Vector, 20ug
L107	Competent Cells Control DNA
L108	pF25K ICE T7 Flexi® Vector
L1081	pF25K ICE T7 Flexi® Vector, 20ug
L109	3.33X Completion Mix
L110	TNT® T7 ICE Master Mix
L1101	TNT®T7 Insect Cell Extract Protein Expression, 10 rxn
L1102	TNT®T7 Insect Cell Extract Protein Expression, 40 rxn
L111	S30 Premix Plus
L1110	S30 T7 High-Yield Protein Expression System, 24 reactions
L1115	S30 T7 High-Yield Protein Expression System, 8 reactions
L112	S30 T7 Control DNA
L1130	E. coli T7-S30 Extract System for Circular DNA
L114	T7-S30 Extract for Circular DNA
L1141	T7-S30 Extract for Circular DNA, 150ul
L1170	TNT® T7 Quick Coupled Transcription/Translation System
L1171	TNT® T7 Quick Coupled System Trial Size
L118	1mM Methionine
L119	BL21 (DE3) pLysS Competent Cells
L1191	BL21 (DE3) pLysS Competent Cells >10x6 cfu/ug, 1ml
L120	BMH71-18 mutS Component Cells
L1201	BMH71-18 mutS, Competent Cells >10x7 cfu/ug, 1ml
L1202	BMH71-18 mutS, Competent Cells, 200ul
L200	JM109 Competent Cells, High Efficiency
L2001	JM109 Competent Cells, High Efficiency, 1ml (5 x 200ul)
L2003	JM109 Competent Cells, High Efficiency, 200ul
L2004	JM109 Competent Cells, High Efficiency, 200ul
L201	HB101 Competent Cells, High Efficiency
L2011	HB101 Competent Cells, High Efficiency, 1ml (5 x 200ul)
L203	Transcend™ Chemiluminescent Substrate A
L204	Transcend™ Chemiluminescent Substrate B
L205	Streptavidin HRP
L2080	TNT® SP6 Quick Coupled Transcription/Translation System
L2081	TNT® SP6 Quick Coupled T/T System, Trial Size
L209	TNT® SP6 Quick Master Mix
L2091	TNT® SP6 Quick Master Mix, 200ul
L216	T7 TNT® PCR Enhancer
L3001	Single Step (KRX) Competent Cells, 5 X 200ul
L3002	Single Step (KRX) Competent Cells, 20 X 50ul