

**Modification Form for Permit BIO-CPRI-0001**

**Permit Holder: Charles Rupar**

**Approved Personnel**

**(Please stroke out any personnel to be removed)**

- Jiahui (James) Liu
- Cathy Regan
- Kathie-Boer

**Additional Personnel**

**(Please list additional personnel here)**

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
<b>Approved Microorganisms</b>	<del>Escherichia coli, Salmonella, Mycobacterium</del>	
<b>Approved Primary and Established Cells</b>	Human (est), Rodent (est), Human (primary) HeLa, 293FT, Rodent (primary) CHO(DG44)	Mouse primary mesenchymal stem cells (MSCs) from C57B16 strain. Neural cells differentiated in vitro from primary MSCs.
<b>Approved Use of Human Source Material</b>		
<b>Approved Genetic Modifications (Plasmids/Vectors)</b>	[bacterial] JM109, Stb13, Mach1[plasmids] pGem-T-Easy, pEGFP-N1, pcDNA 6.2 DEST, plenti6.3-v5-DEST (virus) Replication-deficient, self-inactivating lentiviral derived [vectors] plenti6.3-V5-DEST, pLP1, pLP2,	[plasmids] pLVX-EF1a-IRES-mCherry, pJ204:67966 [vectors containing] LV3-EF-ASA, LV4-A-I-Ch (vector maps and information attached)
<b>Approved Use of Animals</b>	Mus musculus, C57B16, wild-type and ASA knockout	
<b>Approved Biological Toxin(s)</b>		
<b>Approved Gene Therapy</b>		
<b>Approved Plants and Insects</b>		

\* 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.

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: C. A. Ryan

Current Classification: 2 Containment Level for Added Biohazards: 2

Date of Last Biohazardous Agents Registry Form: Jul 9, 2010

Date of Last Modification (if applicable): \_\_\_\_\_

BioSafety Officer(s): Neil Ryan Feb 24/12 J Stanley Feb 24, 2012

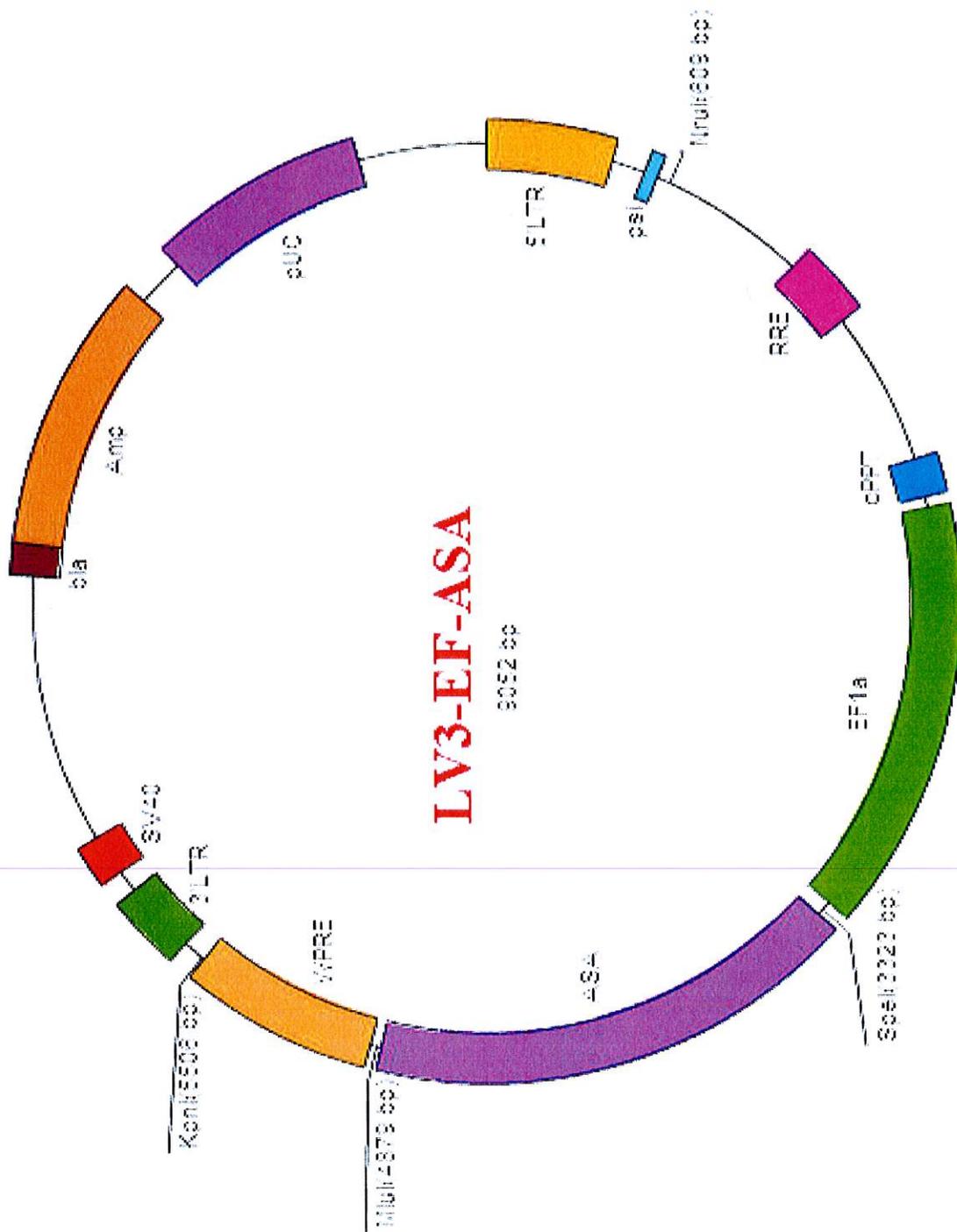
Chair, Biohazards Subcommittee: J Miller Date: Feb 28 2012

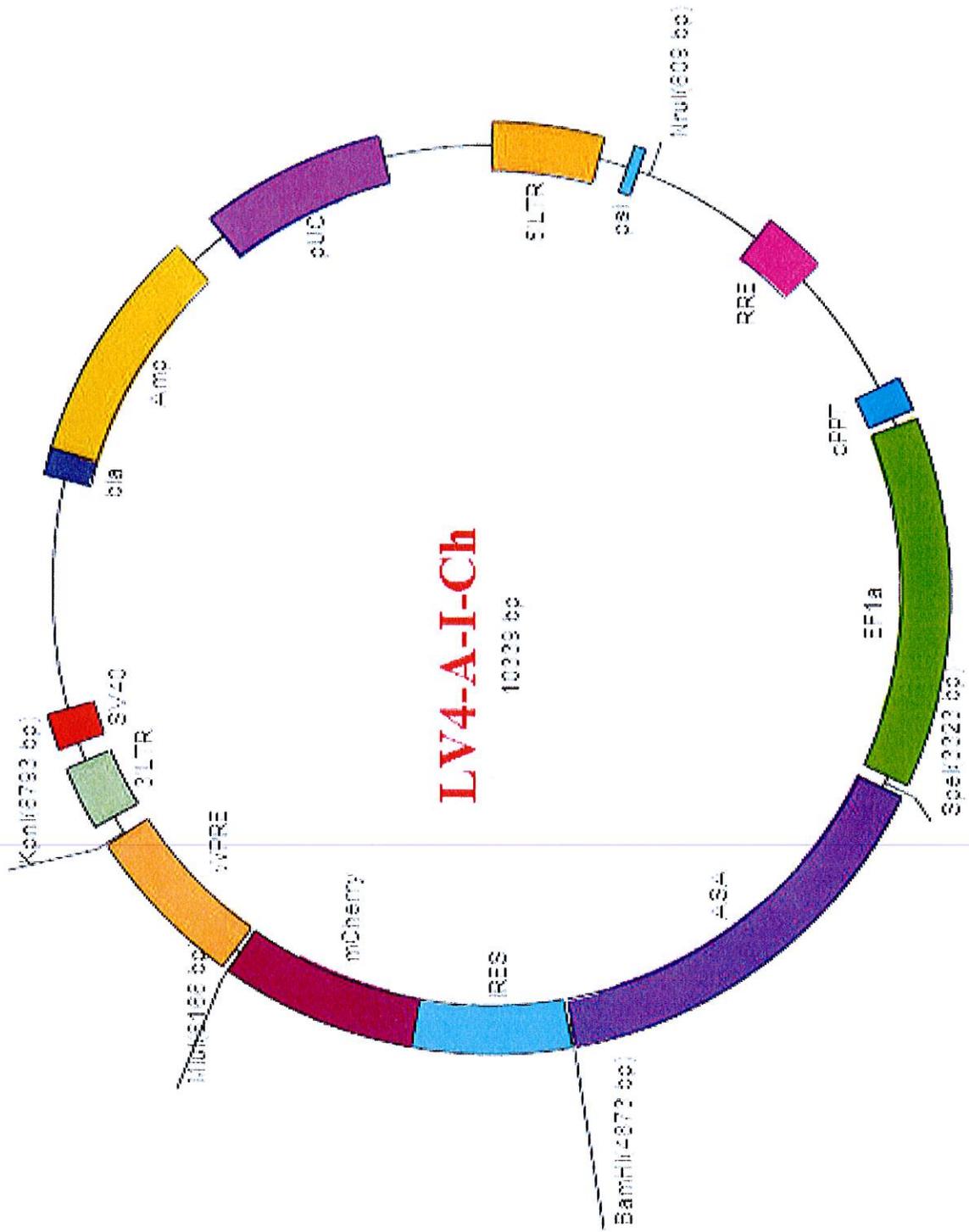
**Biohazardous Agents Registry Form Modifications - RUPAR 2011 - for BIO-CPRI-0001 and BIO-UWO-0233.**

1. The addition of new vector constructs. These constructs contain 3 changes from previously registered vectors:

1. A switch from the CMV promoter to the EF1 $\alpha$  promoter.
2. A switch of fluorescent reporter protein from GFP to mCherry.
3. The use of a codon optimized arylsulfatase A gene. (The gene sequence has been changed, but the protein sequence produced from it is not changed)

2. The use of mouse primary cells in *in vivo* experiments as outlined in animal protocol.





## 11.12.1B - Agent/Material/Drug/Device Information

Species Name	Mouse
1. Agent/Material/Drug/Device Name	Neural Cells
2. Agent/Material/Drug/Device Category	Biological
3. Agent/Material/Drug/Device Class	

### AGENT/MATERIAL/DRUG/DEVICE RELATED QUESTIONS

1. Source of Biological Agent (Please provide website and attach an MSDS or equivalent)	Harvested and cultured from C57Bl6 mice.
2. Biosafety Approval number:	BIO-UWO-0233
3. Is this biological agent on your current Biosafety approval? If not, please submit a protocol modification (See <a href="http://www.uwo.ca/humanresources/biosafety">www.uwo.ca/humanresources/biosafety</a> for information).	Yes
4. Dose/Volume administered, Routes of administration and Frequency of administration:	0.010 mL volume into lateral ventricle of the brain. Single injections per animal.
5. Are you creating genetic modifications using plasmids or viral vectors?	Yes
5.1. If Q.5 is 'Yes', has the Biosafety permit been updated to reflect this modified agent? If 'No', please complete a protocol modification (See <a href="http://www.uwo.ca/humanresources/biosafety">www.uwo.ca/humanresources/biosafety</a> for more information).	Yes
5.2. If Q.5.1. is 'Yes', please describe the expected increase in invasiveness, toxicity or tumourgenicity of the agent in the animal.	No increase. Vector used to transduce these cells is no longer present (through culturing). Additionally, the virus from which the foamy virus vector is derived has been established to be non-pathogenic in humans.
6. Will the biological agent be used according to the Occupational Health and Safety Standard Operating Procedures (SOP) for Use of Biological, Chemical, Radiation and/or other Physical agents with live animals? (please link to	Yes

<a href="http://www.uwo.ca/animal/website/VS/Content/SOPs.htm">http://www.uwo.ca/animal/website/VS/Content/SOPs.htm</a>	
6.1. If Q.6 is 'No', please explain what section(s) will not be followed and why.	
7. Do animals require housing after exposure to the biological agent(s)?	Yes
7.1. If Q.7 is 'Yes', please list all housing and/or imaging facilities.	<b>Remain</b> Animals will be housed in <b>the level 2 Room 6010</b> procedure. room
8. Are animals transported between buildings after exposure to the biological agent(s)?	No
8.1. If Q.8 is 'Yes', please describe the precautions taken during transportation.	
9. Will the agent/material or metabolite be excreted or shed by the animal?	
9.1. If Q.9 is 'Yes', describe the route(s) and duration of shedding. (Please note that bedding will need to be treated as biohazardous by personnel and for disposal. Cages will need to be decontaminated)	No
9.2. If Q.9 is 'No', please explain (Provide documentation if possible).	Cells are too large to "escape" the location of injection. Arylsulfatase A enzyme excreted by these cells is benign.
10. PLEASE INDICATE THE CONTROL MEASURES TO BE TAKEN TO MINIMIZE THE RISK OF EXPOSURE TO ANIMAL FACILITY STAFF:	
10.1. Level 1 Precautions	
10.2. Level 2 Precautions	Yes
10.3. Level 2 Plus Precautions	
10.4. Level 3 Precautions	
10.5. Other - Please provide clarification:	
11. PLEASE INDICATE THE SOPS THAT WILL BE FOLLOWED: (For more information, please see <a href="http://www.uwo.ca/humanresources/facultystaff/h_and_s/biosafety/biosafety_policies.htm">http://www.uwo.ca/humanresources/facultystaff/h_and_s/biosafety/biosafety_policies.htm</a> )	
11.1. ACVS Level 2 Policy (MANDATORY FOR LEVEL 2 PROJECTS.)	Yes

11.2. UWO Biosafety Guidelines and Procedural Manual for Containment Level 1 & 2 Laboratories, see <a href="http://www.uwo.ca/humanresources/biosafety">www.uwo.ca/humanresources/biosafety</a> (MANDATORY FOR ALL PROJECTS).	Yes
11.3. UWO Level 3 Manual (MANDATORY FOR LEVEL 3 PROJECTS)	No
11.4. Viral Vector Policy (MANDATORY FOR PROJECTS INVOLVING VIRAL VECTORS)	<del>NO</del> Yes
11.5. Biosafety Requirements for in vivo and in vitro work (MANDATORY FOR PROJECTS INVOLVING IMAGING AND THE USE OF BIOLOGICAL AGENTS)	<del>Yes</del> No
11.6. Other - Please provide clarification:	<b>The cells will be tested for infectious foamy virus vector upon receipt in our laboratory by qPCR and infectivity of culture media.</b>
12. Please list and describe any additional precautions to be taken.	None.

### Attachments List

File Spec	Description	Created
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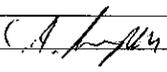
**THE UNIVERSITY OF WESTERN ONTARIO  
 BIOHAZARDOUS AGENTS REGISTRY FORM  
 Approved Biohazards Subcommittee: June 26, 2009  
 Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)**

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, 1<sup>st</sup> 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](mailto: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/](http://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	<a href="mailto:trupar@uwo.ca">trupar@uwo.ca</a>

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	_____
_____	_____
_____	_____

## 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:

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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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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, Sibl3, Mach1)	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	2L	Promega, Invitrogen	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Replication-deficient, self-inactivating lentiviral vectors	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.5L	Made in lab from Invitrogen components	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	Specimens from clinical lab	Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Our mouse colony (C57Bl6 background)	2007-044
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify):	<input type="radio"/> Yes <input checked="" type="radio"/> 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	HeLa, 293FT	293FT - Invitrogen Corp. HeLa - from another local research lab.
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	CHO(DG44)	Dr. L. Chasin, Columbia University
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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	<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid	Clinical laboratory	<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)	Clinical laboratory	<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)	Clinical laboratory	<input type="radio"/> Yes <input checked="" type="radio"/> No		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 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, penti6.3-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/VSFG	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\*



**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 the 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.

SIGNATURE C.A. Ryden

**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 Maile Ryden.

**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. Ryden Date: Feb 26/10

**15.0 Approvals**

UWO Biohazard Subcommittee: SIGNATURE: Susan Koval, Interim Chair  
Date: July 10, 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: Maile Ryden  
Date: April 1, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: J. Stanley  
Date: July 9, 2010

Approval Number: BIO-CPRI-0001 Expiry Date (3 years from Approval): July 8 2013

Special Conditions of Approval:

(NB: in vivo work ~~can~~ is Level 2+ skill)  
In vitro work can be done a Level 2 per modified Viral Vector Policy (approved by Biosafety Committee June 22, 2010); see attached.

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

## Version 5

Approved by Biosafety Committee: June, 2010

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Research with cells transduced with replication competent or defective viral vectors capable of infecting human or animal cells must be carried out in an approved Containment Level 2 (CL2) physical laboratory. This includes, but is not limited to vectors derived from Adenovirus, Adeno-associated virus, lab adapted strains of Vesicular Stomatitis Virus, alpha viruses, measles virus, murine, avian or feline gamma retroviruses (formerly known as type C retroviruses) and herpes simplex virus type I or II. Even though the gamma retroviral vector may be replication defective, endogenous retroviruses residing within the transduced cells *in vitro* or *in vivo* could package the nascent viral RNA as pseudotyped infectious particles. Both amphotropic and xenotropic gamma retroviruses from different species are capable of infecting human cells. How research utilizing replication defective lentiviral vectors is conducted will depend on the nature of the vector and the gene(s) to be transduced. If self-inactivating lentiviral vectors containing a transgene that falls into the category of being a reporter gene (ie GFP, luciferase), a structural gene or a house keeping metabolic enzyme then *in vitro* experiments may be conducted under Containment Level 2 (CL2) physical and operational laboratory conditions. The use of Level 3 (CL3) operational practices (commonly termed CL2+) is still encouraged. However, if the early, non-self-inactivating lentiviral vectors are to be used then, regardless of the nature of the gene to be transduced, CL2 physical laboratory conditions, together with CL3, operational practices (commonly termed CL2+) must be followed. This applies to lentivectors derived from, but not limited to, human immunodeficiency virus (HIV), simian immunodeficiency virus (SIV) and feline immunodeficiency virus (FIV). Researchers are strongly encouraged to use self-inactivating lentiviral vectors and where possible appropriate insulator sequences and tissue or cell type specific promoters. These guidelines also apply to *in vivo* work.

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Research involving a live replication competent or defective viral vector containing a known oncogene, regardless of the type of the viral vector, requires CL3 if the vector is infectious for human cells. Viral vectors expressing genes that are known to be anti-apoptotic, promote cell survival and/or proliferation may also require higher levels of containment but will have to be assessed on a case by case basis by the UWO Biohazards Subcommittee.

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It is recognized that experiments involving direct injection of virus or a virus-transduced cell line into an animal place significant burden on the researchers in order to meet the recommended guidelines. For example, conducting a stereotaxic injection of a viral vector into a targeted area of the brain is generally not possible using conventional laminar flow hoods. Whole animal imaging (MRI, CT, PET or ultrasound, bioluminescence) and flow cytometry of live vector-transduced cells are additional examples where biosafety issues make experimental protocols more difficult. In an effort to help reduce this burden, the following procedures are proposed to provide proof that no virus is being released from transduced cells as a way to reduce the need for CL2 or CL2+ containment.

### Gamma retrovirus or lentivirus vectors:

For experiments that require that cells stably transduced with a gamma retroviral or lentiviral vector be injected into an animal the level of containment can be dropped providing the following conditions can be satisfied:

1. The use of self-inactivating gamma retroviral or lentiviral vectors is strongly advised when available. Commercially available lentiviral vectors are self-inactivating. Most gamma retroviral vectors are not.
2. Once stable viral transductants have been selected/established under the required containment conditions, the engineered cells containing a reporter gene (GFP or luciferase for example), a gene that mediates targeted recombination (Cre or Flip recombinase) or a gene that modifies metabolism but does not affect the cell cycle or proliferation can be tested for the absence of virus production. This can be demonstrated by taking the clarified cell supernatant from the transduced cell line after 5 to 10 cell passages and adding it to cultures of the original uninfected cells or a similar cell line that is highly permissive to viral infection. Reporter gene assays can then be conducted after 48 to 72 hours of culture. However, these types of assays may not be particularly sensitive and should be discussed with the Biohazard Subcommittee in advance. The preferred approach, and that which must be done for all non-reporter gene constructs, is to use quantitative PCR as the confirmatory assay with appropriate standards to confirm assay sensitivity. The assay must be sensitive enough to detect at least one infected cell per  $10^6$  uninfected cells. Alternatively, clarified supernatants from cell passage 5 to 10 can be concentrated by ultracentrifugation and the pellet area extracted in the presence of carrier RNA. Real time qRT-PCR can be conducted with standards to determine if virus is being released from the stably transduced cells. In either case one primer should be derived from the vector sequence and the other from the transgene of interest. If the virus is undetectable in either of these assays, a CL2 or CL2+ cell line could be handled at its original, nontransduced containment level. Animals injected with these reclassified cells could also be handled at their original, nontransduced containment levels. If gamma retrovirus or lentivirus vectors must be injected directly into animals then injections can be conducted in a level 2 room outside of a laminar flow hood provided appropriate personal protective equipment is worn and appropriate decontamination procedures are in place. Once this proof of principle experiment is conducted and submitted to the Biohazard Subcommittee for review, then all subsequent experiments using the same gamma retroviral or lentiviral vector transduced cells can be done under reduced containment. Positive detection of the virus in culture supernatant or as integrated viral DNA from test cells would require maintenance of the virally transduced containment level.

Deleted:

Note that this “dropdown” option does not apply to immunocompromised mice repopulated with primary human or nonhuman primate (NHP), unmodified primary or viral vector modified primary cells. For those mice, the containment must not be lower than CL2 (the standard for handling any primary human material) or CL2+ (the standard for handling NHP material). If the primary cells are known to be infected with a risk group 3 human pathogen, then they must be handled at the containment level appropriate for that pathogen. If the transduced gene is known to promote cell survival or alter cell cycling in favour of proliferation (as in the case of an oncogene), then CL2+ or a higher containment level, determined by a risk assessment made in collaboration with the Biohazard Subcommittee, must be maintained for live viral vector work, especially if the vectors are capable of infecting human cells.

**Adenovirus vectors:**

For animal experiments that require the use of replication competent adenovirus vectors (first generation vectors), level 2 containment must be observed regardless of the transgene to be used. For experiments using 2<sup>nd</sup> or 3<sup>rd</sup> generation replication defective Adenovirus vectors that do not contain an oncogene or genes that promote cell survival and or cell proliferation, direct injection of virus infected cells or direct injection of virus can be done outside a laminar flow hood in an approved level 2 room with personal protective equipment worn once the following proof of principle condition has been satisfied:

Following injection of the animal, bodily fluids such as blood, bronchial lavage, and urine as well as stool should be collected at several time points over the first 14 days post-infection. Quantitative PCR with the use of positive spiking controls and assay sensitivity controls can then be used to demonstrate that the recombinant Adenovirus is not being released from the infected animal. Once this proof of principle experiment is conducted then all following experiments using the same Adenovector can be done under reduced containment conditions and the animals can be returned to CL1 animal housing at the point when the Q-PCR gave reproducible negative results.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

**Adeno-associated virus vectors:**

For experiments using recombinant Adeno-associated virus vectors it is strongly recommended that the vector be generated using a construct that can generate the vector by transfection such that helper virus is not required. For direct animal injection experiments the same proof of principle experiment as described for the Adenovirus vectors must be conducted before lowering of the containment level for animal housing can be considered.

In some cases, the animal can be kept in quarantine at Level 2 containment for a prescribed period of time and then removed to Level 1. To do this, the researcher must provide suitable evidence from the literature regarding an appropriate quarantine period for the specific agent in use. This use of quarantine is approved by the Biohazards Subcommittee on a case-by-case basis.

**Other viral vectors:**

Experiments requiring the use of less commonly used viral vectors will need to be considered by the Biohazard Subcommittee on a case by case basis in consultation with AUS-ACVS.

July  
2010  
(New)

**Subject:** Fwd: Biohazardous Agents Registry Form - Rupar lab in CPRI  
**From:** Jennifer Stanley <jstanle2@uwo.ca>  
**Date:** Wed, 30 Jun 2010 10:55:10 -0400  
**To:** Charles Anthony Rupar <trupar@uwo.ca>, Gail Ryder <Gail.Ryder@LawsonResearch.Com>

Hi Dr. Rupar & Gail -

After the Biosafety Committee meeting you mentioned that you were going to contact the Public Health Agency of Canada about this.  
Please send me an update (for the members of the Biohazards Subcommittee) by next Monday if possible - Tuesday at the latest!

Thanks!  
Jennifer

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

**Subject:** Biohazardous Agents Registry Form - Rupar lab in CPRI  
**Date:** Tue, 22 Jun 2010 09:48:07 -0400  
**From:** Jennifer Stanley <jstanle2@uwo.ca>  
**To:** Charles Anthony Rupar <trupar@uwo.ca>, "avpres@uwo.ca" <avpres@uwo.ca>  
**CC:** Gail Ryder <Gail.Ryder@LawsonResearch.Com>

Hello Dr. Rupar -

According to PHAC, Invitrogen pLenti6/V5 Directional-TOPO cloning kit, catalog number K4950-00, is risk group 2+. Please see the attached information for more information.

Jennifer

PS. If you would like to talk to PHAC directly, here is the information:

Maria Zafer  
Regulatory Technologist/ technologiste en réglementation  
Pathogen Regulation Directorate (formerly Office of Laboratory Security) /  
Direction de la réglementation des agents pathogènes (anciennement le  
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

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

**Subject:** Fwd: Re: Biohazardous Agents Registry Form - Rupar lab in CPRI  
**Date:** Wed, 16 Jun 2010 11:40:14 -0400  
**From:** Tony Rupar <Tony.Rupar@LHSC.ON.CA>  
**To:** Jennifer Stanley <jstanle2@uwo.ca>

yes

>>> Jennifer Stanley <jstanle2@uwo.ca> 2010/06/15 10:05 AM >>>  
Tony  
Do you still use the Invitrogen pLenti6/V5 D-TOPO cloning kit?  
Jennifer

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

**Subject:** Re: Biohazardous Agents Registry Form - Rupar lab in CPRI  
**Date:** Fri, 11 Jun 2010 15:20:34 -0400  
**From:** Tony Rupar <Tony.Rupar@LHSC.ON.CA>  
**To:** Jennifer Stanley <jstanle2@uwo.ca>  
**CC:** Gail Ryder <Gail.Ryder@LawsonResearch.Com>

Hi:

sorry that I omitted the summary in the previous email. Please see the attachment and also the product manual from Invitrogen.

tony rupar

>>> Jennifer Stanley <jstanle2@uwo.ca> 2010/06/10 4:20 PM >>>  
Hello there Dr. Rupar -

New - July 200  
meeting

Tony Rugar

June 10, 2010

### Overview of research program.

Metachromatic leukodystrophy is a fatal autosomal recessively inherited progressive neurodegenerative disorder caused most often by a deficiency of arylsulfatase A. This research project has been to study the disease process in a knockout mouse model and to develop treatments. Treatments based on enzyme replacement therapy and gene transfer therapy have been successful with *in vitro* models and are now succeeding *in vivo* in the mouse model.

### Summary of *in vitro* research that involves lentiviral derived vectors

#### A. Vector preparation.

Replication incompetent gene transfer vectors have been prepared using the Invitrogen third generation ViralPower lentiviral expression system. This system involves the quadruple transfection of the 293FT (Invitrogen) packaging cell line. The vectors are pseudotyped with the VSV-G protein and the transgene expression is driven by the CMV promoter. I have attached the Invitrogen manual that provides details on the biosafety features of the product and the recommendation for working at level 2 or 2 + (depending on risks associated with the transgene). The maximum volume of media from 293FT cells from which vector is prepared is about 240 ml.

Vectors have been prepared with the following transgenes:

1. eGFP (enhanced green fluorescent protein)
2. ARSA-V5 (arylsulfatase A with a V5 epitope tag)
3. TF-ARSA-V5 (Transferrin fused to arylsulfatase A with a V5 epitope tag).
4. ARSA-V5-17 ApoE (arylsulfatase A with a V5 epitope tag fused to a 17 amino acid sequence from apolipoprotein E)
5. eGFP-17ApoE (enhanced green fluorescent protein fused to a 17 amino acid sequence from apolipoprotein E)

Characteristics of the transgenes:

1. eGFP – very widely used as a fluorescent intracellular marker of transduced cells both *in vitro* and *in vivo*. No known toxic or oncogenic risks.
2. ARSA – a ubiquitous (probably present in all mammals and most tissues) lysosomal enzyme that participates in the catabolism of the glycolipid sulfatide. No known toxic or oncogenic risk.
3. TF – transferrin is a ubiquitous (probably present in all mammals) serum protein that transports iron. No known toxic or oncogenic risks.
4. 17ApoE – this a 17 amino acid sequence derived from apolipoprotein E (a component of serum very low density lipoproteins). No known toxic or oncogenic risks.

new  
July 2010  
meeting

## B. Vector utilization

1. Vectors 2, 3, 4 and 5 are used to transduce Chinese hamster ovary (CHO) cells. The transduced CHO cell lines are cultured in serum free media and produce the desired proteins which are purified from the culture media. This work occurs in the CPRI laboratory.
2. Vectors 1 and 2 are used *in vivo* in the mouse knockout model. All of these experiments are done in the level 3 animal facility.

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

**Subject:**Re: Biohazardous Agents Registry Form - Rugar 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>

Page 1 of 2  
New info  
June 3/10

Jennifer,

Dr. Rugar and I met yesterday. Right now his labs do not meet Level 2+ standards but Dr. Rugar 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. Rugar & Gail -

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

Please note, Dr. Rugar, 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](mailto:gail.ryder@lhsc.on.ca)  
Website: [www.lhrionhealth.ca](http://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 BAREF. 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](mailto:Gail.Ryder@LawsonResearch.com)  
Website: [www.lawsonresearch.com](http://www.lawsonresearch.com)

Jennifer Stanley <[jjstanle2@uwo.ca](mailto:jjstanle2@uwo.ca)> 2010/04/08 04:22 PM >>>

Hi Gail

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

Regards  
Jennifer

---

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**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



Ministry of Children  
and Youth Services

Ministère des Services  
à l'enfance et à la jeunesse

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South West Region

district sud-ouest

CPRI  
800 Sanatorium Road  
London ON N6H 3W7

CPRI  
600 chemin Sanatorium  
London (Ontario) N6H 3W7

Date:  
April 13, 2007

Time/Hours/Heure:

To/Destinataire: OHS

From/Expédite CPRI-Biochemical Genetics Laboratory

Name/Nom: Jennifer Stanley

Name/Nom: Cathy Regan

Location/Ville: SLB 296

Location/Ville: London

Telephone/Téléphone: 519-661-2111 X81135

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

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519-661-3420

(519) 858-1063

3

Subject/Sujet:  
From Dr. Tony Rugar

Additional instruction/Directives additionnelles:

Valid consent on file

Not applicable

Verified by: \_\_\_\_\_

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

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Sent by/Envoyé par

Date

file: Rupa

Public Health  
Agency of CanadaAgence 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/bg-\(dmb\)/index.html](http://www.phac-aspc.gc.ca/ols-bsl/bg-(dmb)/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



----- 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) 941-0536



## Containment Level 2+ 3 Operational – Additional Items Checklist

<b>Q.4.</b> <b>(Chapter 3.1.3)</b>	Is a program in place (with appropriate authority to oversee safety and containment practices) for the management of biological safety issues?	<b>MANDATORY</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.19.</b> <b>(Chapter 3.1.3)</b>	Is a containment check performed before entering the containment laboratory (e.g. verify correct readings on the pressure monitoring device)?	<b>MANDATORY</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.20.</b> <b>(Chapter 3.1.3)</b>	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.	<b>MANDATORY</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.24.</b> <b>(Chapter 3.1.3)</b>	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)?	<b>RECOMMENDED</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.25.</b> <b>(Chapter 3.1.3)</b>	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?	<b>MANDATORY</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.34.</b> <b>(Chapter 3.1.3)</b>	Are infectious agents stored inside the containment laboratory?	<b>RECOMMENDED</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> N/A
<b>Q.35.</b> <b>(Chapter 3.1.3)</b>	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.	<b>MANDATORY</b> <input type="checkbox"/> Yes <input type="checkbox"/> No <input type="checkbox"/> 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 ( $\Delta U3$ ) 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  $\Psi$  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).

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*Continued on next page*

## Biosafety Features of the System, Continued

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### 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," 4<sup>th</sup> 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>

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### 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.

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**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)  
 800/955-6288

**NON-EMERGENCY INFORMATION:**

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, therapeutic, 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

MATERIAL SAFETY DATA SHEET

VIRAPOWER PKG. MIX 195 UG	Page	2 of 8
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	Printed	1/30/03

**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.

MATERIAL SAFETY DATA SHEET Page 3 of 8  
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 Printed 1/30/03

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

**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

MATERIAL SAFETY DATA SHEET

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#### 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	ACGIH TWA
TRIZMA BASE	(ppm) Not established.	(ppm) 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.

MATERIAL SAFETY DATA SHEET

VIRAPOWER PKG. MIX 195 UG INVITROGEN CORPORATION MSDS ID: 442050	Page 5 of 8 Revised 1/30/03 Replaces (None) Printed 1/30/03
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**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 alkalis. 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.

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**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:

MATERIAL SAFETY DATA SHEET Page 7 of 8  
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 Printed 1/30/03

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

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  
 TRIZMA BASE Number  
 Not established.

16. OTHER INFORMATION

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

MATERIAL SAFETY DATA SHEET

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

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 Replaces (None)  
 Printed 1/30/03

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.

<b>1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING</b>
---

**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

<b>2. COMPOSITION/INFORMATION ON INGREDIENTS</b>
--

**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.

<b>3. HAZARDS IDENTIFICATION</b>
----------------------------------

**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 recommended. 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 contain 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 PARTICULAR PURPOSE.

**End of Safety Data Sheet**

**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

<b>Proper shipping name</b>	Not classified as dangerous in the meaning of transport regulations
<b>Hazard Class</b>	No information available
<b>Subsidiary Class</b>	No information available
<b>Packing group</b>	No information available
<b>UN-No</b>	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 contain 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 PARTICULAR PURPOSE.

**End of Safety Data Sheet**

## Project Summary – Rugar 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

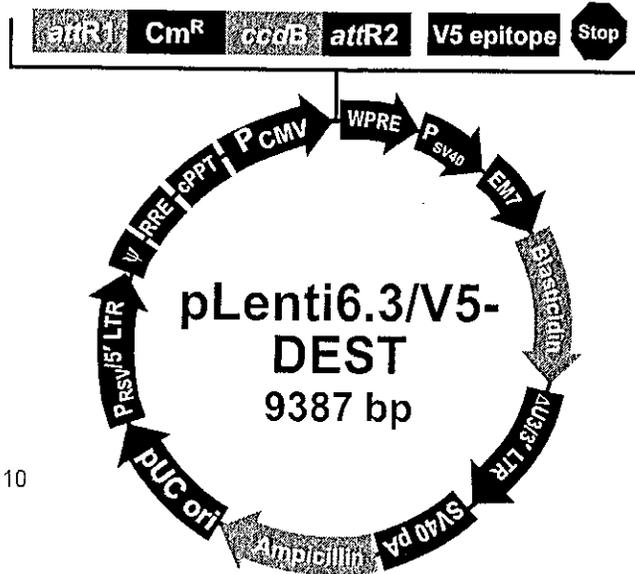
### 3. HeLa – to titer vector

#### 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.

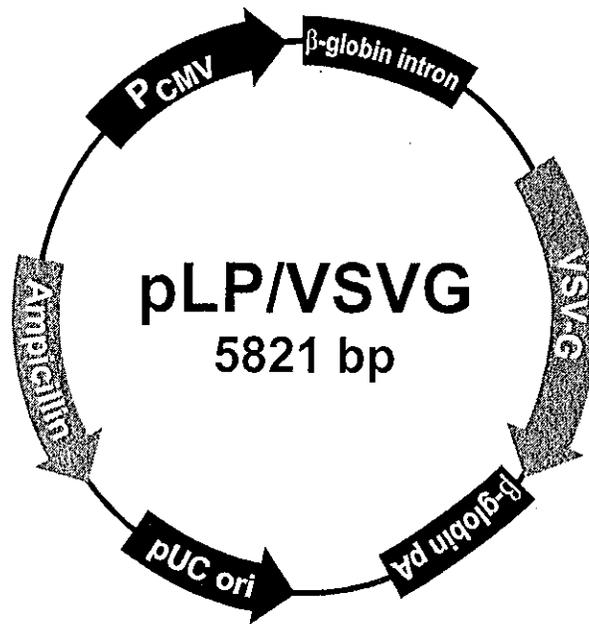
#### Human tissues:

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 ( $\psi$ ) 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
- attR1* site: bases 2568-2692
- Chloramphenicol resistance gene (*Cm<sup>R</sup>*): bases 2801-3460
- ccdB* gene: bases 3802-4107
- attR2* 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
- $\Delta$ U3/3' LTR: bases 5907-6141
- $\Delta$ U3: 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



**Comments for pLP/VSVG**  
5821 nucleotides

CMV promoter: bases 1-747

TATA box: bases 648-651

Human  $\beta$ -globin intron: bases 880-1320

VSV G glycoprotein (VSV-G): bases 1346-2881

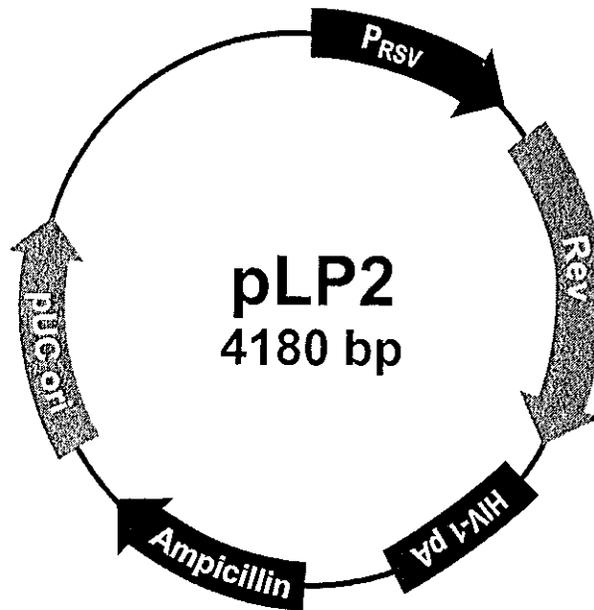
Human  $\beta$ -globin polyadenylation signal: bases 3004-3769

pUC origin: bases 3927-4600 (C)

Ampicillin (*bla*) resistance gene: bases 4745-5605 (C)

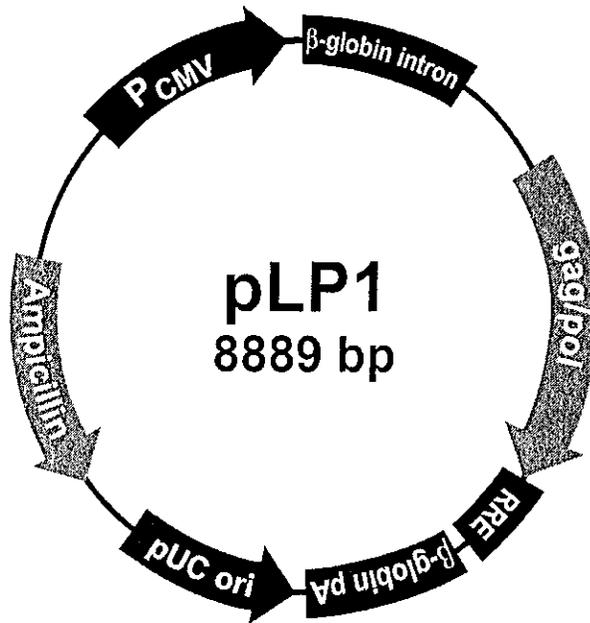
*bla* promoter: bases 5606-5704 (C)

C=complementary strand



**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



**Comments for pLP1**  
8889 nucleotides

CMV promoter: bases 1-747

TATA box: bases 648-651

Human  $\beta$ -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

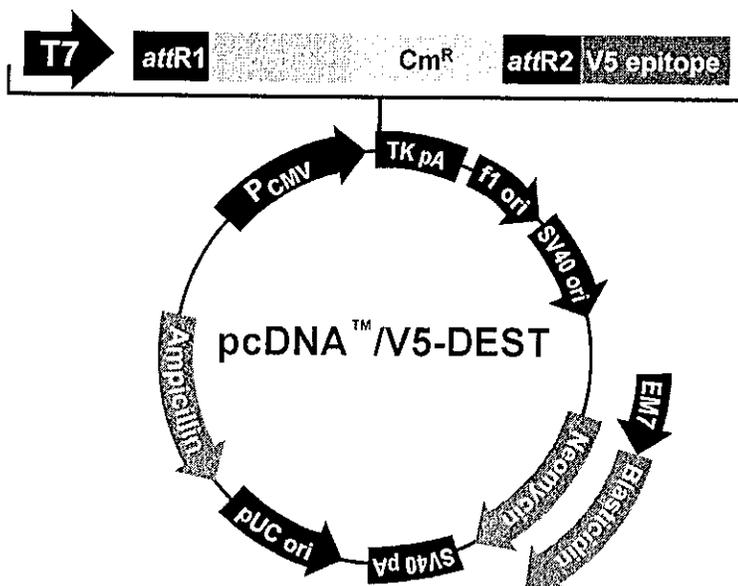
Human  $\beta$ -globin polyadenylation signal: bases 6072-6837

pUC origin: bases 6995-7668 (C)

Ampicillin (*bla*) resistance gene: bases 7813-8673 (C)

*bla* promoter: bases 8674-8772 (C)

C=complementary strand



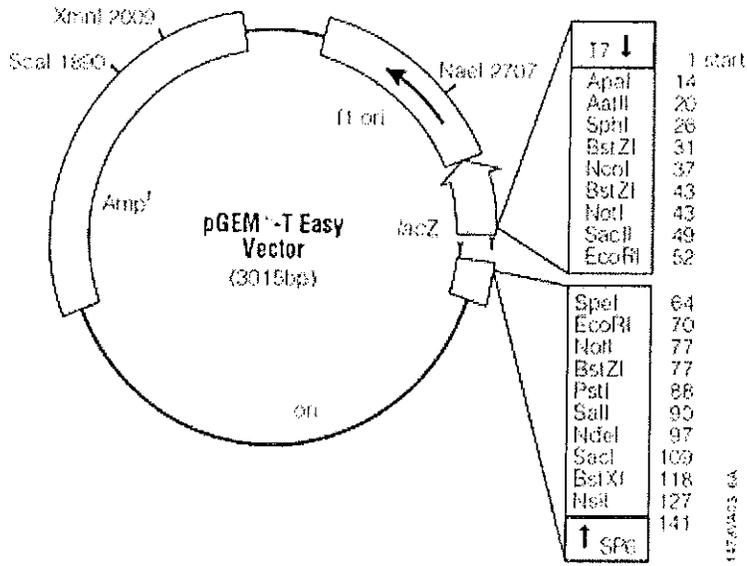
**Comments for:**

	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
attR1 site:	911-1035	911-1035
ccdB gene (c):	1464-1769	1464-1769
Chloramphenicol resistance gene (c):	2111-2770	2111-2770
attR2 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 ( <i>bla</i> ) resistance gene (c):	6715-7575	6345-7205
<i>bla</i> promoter (c):	7576-7674	7206-7304

(c) = complementary strand

pgem-t easy vector system i

a1360



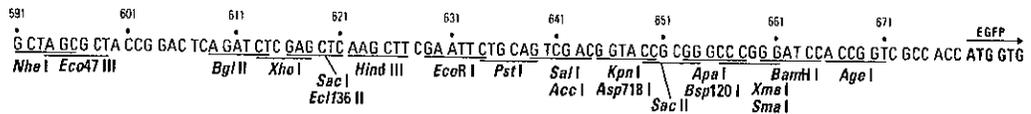
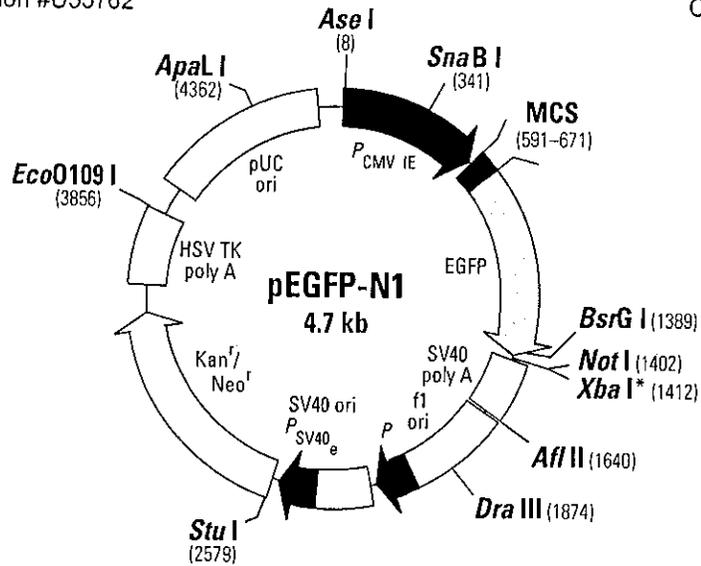
Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA  
608-274-4330

**pEGFP-N1 Vector Information**

GenBank Accession #U55762

PT3027-5

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*<sup>-</sup> 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<sub>CMV IE</sub>*) 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 (Neo<sup>r</sup>), 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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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 promoter for expression of *Kan<sup>r</sup>* 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. & Tsujii, 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.



### 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

<b>Proper shipping name</b>	Not classified as dangerous in the meaning of transport regulations
<b>Hazard Class</b>	No information available
<b>Subsidiary Class</b>	No information available
<b>Packing group</b>	No information available
<b>UN-No</b>	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 contain 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 PARTICULAR PURPOSE.

End of Safety Data Sheet



# Promega

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.**

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February 2002

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

Product code 500324  
 Product name **MACH 1 ONE SHOT COMP CELL**

**Contact manufacturer**  
 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**  
 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 PARTICULAR PURPOSE.

End of Safety Data Sheet



**Promega**

Promega Corporation  
 2800 Woods Hollow Road  
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 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/EEC, 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 Packagene® System
- K315 Packagene® Lambda DNA Packaging System, 11 Ext
- K3152 Packagene® Lambda DNA Packaging System, 6 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	<b>JM109 Competent Cells, High Efficiency, 1ml (5 x 200ul)</b>
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 T7 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