

Modification Form for Permit BIO-LRCC-0017

Permit Holder: Trevor Shepherd

PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOLOGICAL AGENTS.

PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOLOGICAL AGENTS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF.

Approved Personnel

(Please stroke out any personnel to be removed)

~~Jason Reed~~

Rohann Correa

Teresa Peart

Additional Personnel

(Please list additional personnel here)

Samah Rafahi

Jessica Tony

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	vMyxGFP, DH5alpha, Recombinant hAdV	
Approved Primary and Established Cells	Primary: [human] ovarian cancer ascites fluid, [rodent] ovarian surface epithelial cells. Established: [human] SkOV3, SkOV3-ip1, OVCA429, OCC-1, HeyC2, OVCAR3, CaOV3, 293T, 293A, IOSE80, vOSE-14,	
Approved Use of Human Source Material	Human blood (whole) or other Body Fluid, [Unpreserved] Human Organs or Tissues	
Approved Genetic Modifications (Plasmids/Vectors)	[plasmids]: pcDNA3.0, pSCA. [vectors]: Recombinant Ad5 (AdEasy), E1A Oncogene	MSCV influenza Pathogen (plasmid)
Approved Use of Animals	Mus musculus	
Approved Biological Toxin(s)		
Approved Gene Therapy		

Approved Plants and
Insects

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

Signature of Permit Holder: _____

Current Classification: 2

Containment Level for Added Biohazards: /

Date of Last Biohazardous Agents Registry Form: Jun 23, 2011

Date of Last Modification (if applicable): _____

BioSafety Officer(s)*: _____

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

Chair, Biohazards Subcommittee: _____

Date: _____

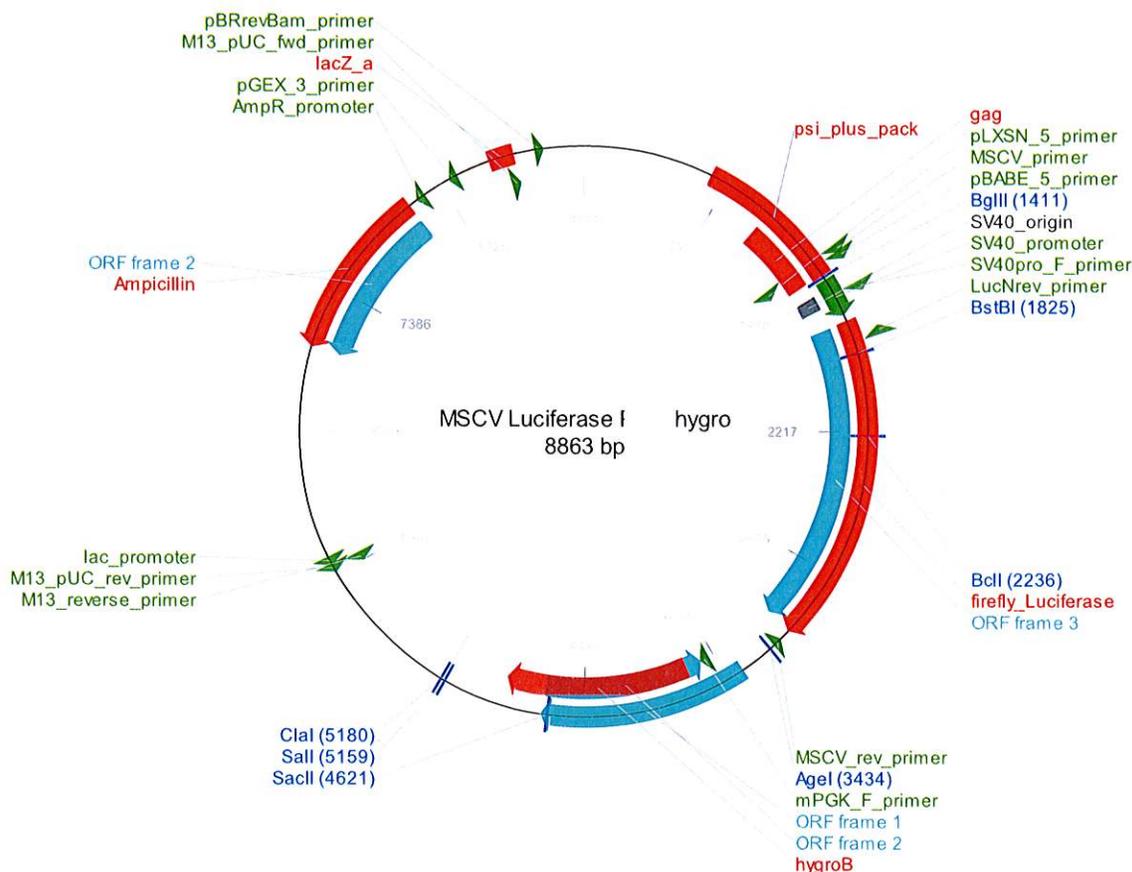


[Browse](#) > [Scott Lowe](#) > [Low Lab Plasmids](#) > MSCV Luciferase PGK-hygro

Plasmid 18782: MSCV Luciferase PGK-hygro

Gene/insert name: None
 Vector backbone: MSCV Luciferase PGK Hygro
[\(Search Vector Database\)](#)
 Vector type: Mammalian Expression, Retroviral, Luciferase
 5' sequencing primer: MSCV [List of Sequencing Primers](#)
 Bacterial resistance(s) Ampicillin
 Growth strain(s) DH5alpha
 Growth temperature (°C): 37
 High or low copy: High Copy
 Selectable markers: Hygromycin
 Sequence: [View sequences \(2\)](#)
 Principal Investigator: Scott Lowe
 Terms and Licenses: [MTA](#)
[Luciferase Limited Use Label License](#)

Addgene has sequenced a portion of this plasmid for verification. Click [here](#) for the sequencing result.



Feature Name	Start	End
psi_plus_pack	654	1404
gag	990	1398

MSCV_primer	1333	1355
pLXSN_5_primer	1333	1355
pBABE_5_primer	1372	1388
SV40_promoter	1424	1626
SV40_origin	1471	1548
SV40pro_F_primer	1533	1552
firefly_Luciferase	1656	3308
LucNrev_primer	1740	1722
MSCV_rev_primer	3382	3359
mPGK_F_primer	3756	3775
hygroB	3856	4864
M13_reverse_primer	5922	5904
M13_pUC_rev_primer	5943	5921
lac_promoter	5986	5957
Ampicillin	7929	7069
AmpR_promoter	7999	7971
pGEX_3_primer	8180	8158
lacZ_a	8510	8379
M13_pUC_fwd_primer	8493	8515
pBRrevBam_primer	8643	8662

ORF	Start	End
ORF frame 3	1656	3308
ORF frame 1	3604	4656
ORF frame 2	4647	3769
ORF frame 2	7929	7069

Enzyme Name	Cut
BglII	1411
BstBI	1825
BclI	2236
AgeI	3434
SacII	4621
Sall	5159
Clal	5180

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 18782" in your Materials and Methods section.

Plasmid: MSCV-luciferase PGK-hygro

Use: This plasmid will be used to transfect cell lines to generate cells that stably-express luciferase after selection using hygromycin antibiotic.

Storage: Plasmid stocks will be stored at -20C.

Disposal: Any plasmid waste will be disposed of by standard method of discarding in sealed tube into biohazard waste (yellow bag in biohazardous waste box) in the lab. Stericycle collects sealed and labeled waste containers for autoclave/incineration followed by disposal.

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

Subject: Fwd: Re: Fwd: Fwd: BIO-LRCC-0017 Form (Shepherd) - Approval

Date: Wed, 01 Aug 2012 16:35:50 -0400

From: Jennifer Stanley <jstanle2@uwo.ca>

To: Jamie.Gibbings@LHSC.ON.CA, tshephe6@uwo.ca

Hi there

I can put it on the agenda for the Committee if you like.

I do want to advise you that it will unlikely be approved, due to the fact that it is so hard to read/illegible.

Please let me know how you want me to proceed.

Regards

Jennifer

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	<u>Trevor Shepherd</u>
DEPARTMENT	<u>Cancer Research Laboratory Program</u>
ADDRESS	<u>790 Commissioners Rd E A4-921</u>
PHONE NUMBER	<u>519-685-8500 56347 (office) 53626 (lab)</u>
EMERGENCY PHONE NUMBER(S)	<u>519-349-2057 (home)</u>
EMAIL	<u>tshephe6@uwo.ca</u>

Location of experimental work to be carried out: Building(s) **LHSC/LRCP Room(s) A4-921, -908**

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: (1) **CCSRI** (2) **LRCP Small Grants**
GRANT TITLE(S): (1) **Implications of activated BMP signalling and ID1/ID3 function in ovarian cancer pathogenesis**; (2) **Myxoma virus mediated oncolysis as a novel therapeutic for epithelial ovarian cancer**

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

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
Teresa Peart	Teresa.peart@gmail.com	Registered for Aug. 24, 2011
Rohann Correa	Rcorrea4@uwo.ca	October 2008
Jason Reed	Jreed7@uwo.ca	September 2009

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Cell lines

Numerous established cell lines are used in my laboratory including SkOV3, SkOV3-ip1, OVCA429, OCC-1, HeyC2, OVCAR3, CaOV3, 293T, 293A, IOSE80, vOSE-14, which are all human cell lines, and 4306, MOSE-RM, MASC2, which are mouse cell lines, and BGMK cells which are non-human primate cells. Frozen vials are stored at -150C until use. They are grown at 37C in 5% CO2 in humidified incubators in our cell culture room (LRCP A4-908). Any waste from culture of these cells disposed of into biohazardous waste containers kept in the cell culture room, which are then sealed to be autoclaved/incinerated.

Primary Cell Culture

Ovarian cancer cells are cultured directly from patients treated at the LHSC and LRCP hospitals. All processing and culture occurs in the cell culture room LRCP A4-908. Excess patient fluids/tissues are bleached and disposed of in sealed biohazardous waste containers for pickup and autoclave/incineration. Frozen vials are stored at -150C until use. Any waste from culture of these materials is disposed of into biohazardous waste containers kept in the cell culture room, which are then sealed to be autoclaved/incinerated.

Viruses

Two types of viruses are currently used in the laboratory in cell culture experiments: recombinant human adenovirus and myxoma virus. All adenovirus constructs are derived from Ad5 serotype and have mutations that render them non-infectious and non-replicating. No adenovirus vector expresses an oncogene or disease-causing agents.

The myxoma virus currently being used was derived by our collaborator Dr. Grant McFadden (U. of Florida Gainesville). This virus has mutations that make it less pathogenic to its natural host the European rabbit. It is non-pathogenic to humans. Our lab has had PHAC approval to import vials of the virus from the USA, and we now routinely make our own virus in the lab using the BGMK cell line.

All viruses are stored at -80C.

All virus work is performed in the Level 2 room A4-908 and in a Class A/B2 biological safety cabinet.

All unused virus is bleached and transferred directly to biohazardous waste in the cell culture room A4-908 to be subsequently sealed and autoclaved/incinerated.

Transformed bacteria

Our lab uses E. coli DH5alpha cells to transform plasmids for routine molecular biology and DNA cloning strategies. The standard vectors we use are the pSCA vector (Stratagene) for cloning PCR products, and pcDNA3.0 for expressing genes in mammalian cells. No oncogene is cloned or expressed in cells using these vectors.

Stocks of transformed bacteria are kept at -80C until use.

Temporary storage of transformed bacterial cultures are kept at 4C in A4-921

Unused bacteria are bleached and disposed of directly into biohazardous waste containers in A4-921 which are sealed and picked up for autoclave/incineration.

Implications of activated BMP signalling and ID1/ID3 function in ovarian cancer pathogenesis

Project summary:

Ovarian cancer has one of the highest death rates of all cancers in women due primarily to unreliable early detection and ineffective drugs to treat the disease after it has spread. Essential to developing better detection methods and drugs is the discovery of critical factors (ie, proteins) that drive the development of ovarian cancer. Dr. Shepherd's research focusses on a group of proteins (bone morphogenetic proteins - BMPs) that allow cells to communicate with each other and regulates the behaviour of ovarian cancer cells isolated from patients.

Previous research:

Dr. Shepherd has shown that the BMPs are produced by ovarian cancer cells and that they feedback on these cells to control their shape, movement and proliferation; characteristics that determine the aggressiveness of cancer cells. As the new Translational Ovarian Cancer Scientist, he is developing novel experimental models utilizing ovarian cancer patient tumour cells to determine how the BMPs, and two genes controlled by BMPs, specifically ID1 and ID3, function to regulate the initiation and progression of ovarian cancer. Dr. Shepherd, with co-investigator Dr. DiMattia, form the basic scientist component of the Translational Ovarian Cancer Research Program at the London Health Sciences Centre and work with gynaecologic oncology surgeons to procure and maintain ovarian cancer patient cells to use in laboratory studies.

Project description:

How BMP signals and ID1 and ID3 genes regulate the behaviour of normal cells of the ovary and ovarian cancer cells will be studied using three new "model systems" being developed in the Shepherd laboratory:

Research Aim #1: Collect ovarian cancer cells from patients and grow them in the lab under conditions that imitate how they grow in ovarian cancer patients as 3D aggregates or spheroids—direct analysis of ovarian cancer cells from patients using this model system will provide more accurate results and thereby generate clinically-relevant insights into the disease;

Research Aim #2: Establish ovarian tumours on the surface of shell-less chick embryos as a model of ovarian cancer growth—this will be a unique opportunity to develop an innovative "bioassay" to assess how BMPs regulate tumour growth and blood supply and for future testing of new drugs that can be used to treat ovarian cancer patients;

Research Aim #3: Develop genetically-altered mice with higher than normal levels of ID1, as Dr. Shepherd has reported for ovarian tumours. We expect these mice will mimic early stages of ovarian cancer—understanding the initial molecular changes that can cause normal cells of the ovary to become cancer cells is indispensable to develop new strategies for prevention and early detection of ovarian cancer.

Impact and relevance:

These three Research Aims will define the role of BMP signals and ID1 and ID3 genes in both early and late stages of ovarian cancer. Most importantly, these studies serve to lay the foundation of the newly-established Translational Ovarian Cancer Program in London. To that end, Dr. Shepherd's studies will develop innovative experimental models for future pre-clinical research endeavours for early detection and to identify and test important drug targets for treating women diagnosed with ovarian cancer.

Myxoma virus-mediated oncolysis as a novel therapeutic for epithelial ovarian cancer

Every year in Canada around 1700 women die from ovarian cancer, and this is almost entirely due to the fact that current therapy for late-stage aggressive disease is not very effective. Over the last decade or so, several groups have been engineering viruses to target and kill cancer cells, while sparing normal cells and tissues.

This approach may also hold promise for treating ovarian cancer; thus, our lab is proposing to test a virus first identified in rabbits: *Myxoma virus*. Myxoma virus has the unique ability to quickly enter cancer cells and kill them when the cancer cells have mutated or altered signal molecules. We have already performed initial experiments in the lab demonstrating that Myxoma virus is able to infect and kill ovarian cancer cells isolated and grown from several different patients. Thus, it is imperative that further experiments be performed to directly evaluate some of the "molecular mechanisms" involved in the process of Myxoma virus infection, and to thoroughly test its effectiveness as a new therapy using several different pre-clinical models of ovarian cancer. It is our hope that this research project will rapidly uncover a new and innovative approach to treat women with this devastating disease.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? YES NO

If YES, please give the name of the species. *Myxoma virus*

What is the origin of the microorganism(s)? *Dr. G. McFadden, U of Florida Gainesville*

Please describe the risk (if any) of escape and how this will be mitigated: *only infectious in European rabbits; the virus strain we use has mutation in gene to render less pathogenic in rabbits*

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

Import permit is attached

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
<i>vMyxGFP</i>	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<i>0.1</i>	<i>Dr. G. McFadden</i>	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
<i>DH5alpha</i>	<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	<i>0.5</i>	<i>Stratagene</i>	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
<i>Recombinant hAdV</i>	<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	<i>0.01</i>	<i>Qbiogene, Vector Biolabs</i>	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <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"/> 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	<i>Ovarian cancer ascites fluid</i>	<i>Not applicable</i>
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>Ovarian surface epithelial cells</i>	<i>2007-022</i>
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

E. coli DHS alpha

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	SkOV3, SkOV3-ip1, OVCA429, OCC-1, HeyC2, OVCAR3, CaOV3, 293T, 293A, IOSE80, vOSE-14	ATCC; G. Mills MD Anderson Cancer Centre; B. Vanderhyden U of Ottawa; C. Conover; Mayo Clinic; N. Auersperg UBC
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	4306, MOSE-RM, MASC2	D. Dinulescu Brigham & Womens Hospital; B. Vanderhyden U of Ottawa
Non-human primate	<input checked="" type="radio"/> Yes <input type="radio"/> No	BGMK	G. McFadden U of Florida
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

MSDS appear to be unavailable for the following cell lines, but the original sources for each individual line are listed: SkOV3-ip1, HeyC2, OCC-1 (orig. source: Mills), OVCA429, MOSE-RM, MASC2 (source: Vanderhyden), IOSE80 (source: Auersperg), vOSE-14 (source: Conover), 4306 (source: Dinulescu) & BGMK (source: McFadden).

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required 1 2 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 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	LHSC/LRCP	<input type="radio"/> Yes <input checked="" type="radio"/> Unknown		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)	LHSC	<input type="radio"/> Yes <input checked="" type="radio"/> Unknown		<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from

				transformation or tranfection
DH5alpha	pcDNA3.0 pSCA	Invitrogen Stratagene	ALK3QD, ID1, ID3	Only ALK3QD virus renders phenotypic changes to cells, including cell adhesion, motility, and differentiation; after transfection with ID1 and ID3 no phenotype has been observed

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

** Please attach a plasmid map.

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 from transduction
Human adenovirus	Recombinant Ad5 (AdEasy)	Qbiogene	Green fluorescent protein (GFP), bacterial beta-galactosidase (lacZ), Cre recombinase, ALK3QD, ID1 and ID3	ALK3QD virus renders phenotypic changes to cells, including cell adhesion, motility, and differentiation; Ad-Cre is used for in vivo mouse experiments to turn on the ALK3QD gene in specific tissues (i.e. ovary); all other viruses listed produce protein products that elicit no phenotype

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ 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

E1A oncogene has been deleted from the human adenovirus

- ◆ 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

E1A oncogene has been deleted from the human adenovirus

4.6 Will virus be infectious to humans or animals?

YES NO

The recombinant human adenovirus that is being used has the ability to transduce cells, but cannot complete lytic infection without the E1A gene product being supplied in trans (i.e. when infecting 293 packaging cells)

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

The adenoviruses we are using do not contain known oncogenes and therefore should be used at Containment Level 2 in our standard cell culture room (LHSC-LRCP A4-908).

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* (mouse)

6.3 AUS protocol # 2007-022 (DiMattia is PI and Shepherd is co-PI)

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: Ad-GFP, Ad-Cre NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

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

8.3 What is the LD₅₀ (specify species) of the toxin _____

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

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? YES, Please provide details:_____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

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

10.0 Plants

10.1 Do you use plants? 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 YES, Please attach the CFIA permit & 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** NO
If no, please proceed to Section 12.0

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 # _____ 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE 

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.

O1 O2 X2+ O3

13.2 Has the facility been certified by OHS for this level of containment?
X YES, permit # if on-campus _____
O NO, please certify
O NOT REQUIRED for Level 1 containment

certified Dec. 2010
by GAIL RYDER
Gail Ryder

14.0 Procedures to be Followed

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

SIGNATURE

Date: December 17, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
Standard operating procedures for these agents (i.e. use and disposal) at each containment level are sufficient to reduce risks to health and safety of personnel.

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:
Occupational Health & Safety at the LHSC will be contacted immediately; however, the agents to be used are not infectious to humans and are not zoonotic in nature

15.0 Approvals

1) UWO Biohazards Subcommittee:

SIGNATURE: [Signature]
Date: 23 June 2011

2) Safety Officer for the University of Western Ontario

SIGNATURE: J Stanley
Date: June 17/11

3) Safety Officer for Institution where experiments will take place (if not UWO):

SIGNATURE: Gail Ryder
Date: Jan 5, 2011

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

Special Conditions of Approval:

• Animal work is level 2. Waste must be treated as biohazardous for 2 weeks post-injection due to the possibility of viral shedding. See attached info.



**Policy on Research Utilizing Virus Vector Transduced Cells or
Virus Infection of Animals
Version 5**

Approved by Biosafety Committee: June, 2010

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. The nature of the vector and the gene(s) to be transduced will determine how research utilizing replication defective lentiviral vectors is conducted. If self-inactivating lentiviral vectors are used and the transgene being transduced falls into the category of being a reporter gene (ie GFP, luciferase), a structural gene, or a housekeeping 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 any of 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.

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 or 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.

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 permissible 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.

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 2nd or 3rd 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.



Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-03324-4
ORIGINAL
2009/07/23
year/mo/day
année/mois/jour

IMPORT PERMIT

PERMIS D'IMPORTATION

Page 1 of/de 3

THIS PERMIT IS ISSUED PURSUANT TO/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A:

THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

<u>Importer/Importateur</u> LONDON HEALTH SCIENCES CENTRE LONDON REGIONAL CANCER PROGRAM 790 COMMISSIONERS ROAD EAST LONDON, ONTARIO N6A4L6 Applicant Name: TREVOR G. SHEPHERD Phone: 519-685-8500 EXT 56347 Fax: 519-685-8673 Email: TSHEPHE6@UWO.CA		<u>Exporter/Exportateur</u> UNIVERSITY OF FLORIDA 1600 S.W. ARCHER ROAD DEPARTMENT OF MOLECULAR GENETICS & MICROBIOLOGY GAINESVILLE, FLORIDA UNITED Contact: Dr. Grant McFadden / Sherin Smallwood Phone: (352) 273-6852 Fax: (352) 273-6849	
<u>Quarantine/Destination/Quarantaine</u>		<u>Producer/Producteur</u>	
<u>Valid/Valide</u>	<u>from/du</u>	<u>to/au</u>	<u>Country of Origin/ Pays d'Origine</u>
	2009/07/23 year/month/day année/mois/jour	2010/07/31 year/month/day année/mois/jour	UNITED STATES
<u>For the entry of/ Pour l'entrée de:</u>		Single shipment/Chargement simple <input checked="" type="checkbox"/> Multiple shipments/Chargements multiples	
<u>Place of entry into Canada/Lieu d'entrée au Canada:</u> ALL REGULATED PORTS			
FOR THE IMPORTATION OF:/POUR L'IMPORTATION DE: (Description of things(s)/Description de la ou des choses) 1. Product Description: MYXOMA VIRUS - PLEASE SEE ADDITIONAL CONDITIONS AT THE END OF THIS PERMIT. (TO BE USED IN VITRO ONLY IN ROOM A4-908, CANCER RESEARCH LABORATORY, LONDON HEALTH SCIENCES CENTRE, LONDON, ON) Proposed End Use: "In Vitro" Scientific Name: Biocontainment Level: 2			
A PERSON WHO IMPORTS A THING UNDER THIS PERMIT SHALL COMPLY WITH ALL THE CONDITIONS SET OUT HEREIN/TOUTE PERSONNE QUI IMPORTE UNE CHOSE EN VERTU DE CE PERMIS DEVRA RESPECTER TOUTES LES CONDITIONS DÉCRITES CI-DESSOUS			

Selected Conditions / Conditions Choies

MYXOMA VIRUS - PLEASE SEE ADDITIONAL CONDITIONS AT THE END OF THIS PERMIT.

(TO BE USED IN VITRO ONLY IN ROOM A4-908, CANCER RESEARCH LABORATORY, LONDON HEALTH SCIENCES CENTRE, LONDON, ON)

- The original or a copy of the signed original of this permit and any other necessary import / export documentation pertaining to the shipment of animal(s) or thing(s) must be provided for inspection at the first port of entry or to a Canadian Food Inspection Agency Import Service Center.
- The conditions in this permit can only be changed or amended by a CFIA inspector. Any change to the permit by an unauthorized person will render the permit invalid.
- The imported material must be packaged in appropriate shipping containers to prevent accidental spillage of contents during shipping. Importers should be aware of their obligations under Transport Canada's regulations concerning transportation of dangerous goods.



Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-03324-4
ORIGINAL
2009/07/23
year/mo/day
année/mois/jour

IMPORT PERMIT

PERMIS D'IMPORTATION

Page 2 of/dc 3

THIS PERMIT IS ISSUED PURSUANT TO:/CE PERMIS EST DÉLIVRÉ CONFORMÈMENT A:

THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur

LONDON HEALTH SCIENCES CENTRE

LONDON REGIONAL CANCER PROGRAM

790 COMMISSIONERS ROAD EAST

LONDON, ONTARIO

N6A4L6

Applicant Name: TREVOR G. SHEPHERD

Phone: 519-685-8500 EXT 56347 Fax: 519-685-8673

Email: TSHEPHE6@UWO.CA

Exporter/Exportateur

UNIVERSITY OF FLORIDA

1600 S.W. ARCHER ROAD

DEPARTMENT OF MOLECULAR GENETICS & MICROBIOLOGY

GAINESVILLE, FLORIDA

UNITED

Contact: Dr. Grant McFadden / Shcrin Smallwood

Phone: (352) 273-6852 Fax: (352) 273-6849

Selected Conditions / Conditions Choies (Continued/Suite)

4. All infectious material must be handled in appropriate animal pathogen containment level 2 facilities as described in Containment Standards for Veterinary Facilities, 1996, AAFC publication no. 1921.
5. The material authorized for importation by this permit is to be used in in vitro studies ONLY and must not be introduced into laboratory, domestic or wild animals (including birds or fish) unless written authorization is obtained from the Canadian Food Inspection Agency.
6. The animal(s) or thing(s) imported under this permit must NEVER be removed from the premises of destination listed on this permit, even after the animals have been released from their post-import quarantine, unless written authorization is obtained from the Canadian Food Inspection Agency.
7. Upon completion of the tests or experiments, the imported material as described on this permit and any derivatives thereof must be autoclaved, incinerated or alternatively disposed of in a manner approved by an inspector of the Canadian Food Inspection Agency.
8. Records pertaining to the imported product's use, storage and disposal must be maintained for two (2) years following importation. These records must be made available for inspection by the Canadian Food Inspection Agency upon request.
9. The importer is responsible for all costs incurred or associated with any testing or treatment of the animal(s) or thing(s) that may be required under the import permit or under the authority of the Health of Animals Act or the Health of Animals Regulations. The importer shall pay all fees for services required in respect of the importation under the National Animal Health Program Cost Recovery Fees Regulations in place at the time of importation.
10. Consideration of an application necessary for issuance of a permit to import the described animal or thing is subject to Class 1 fees.
11. The issuance of this permit does not relieve the owner or the importer of the obligation to comply with any other relevant federal, provincial or municipal legislation or requirement.
12. Failure to comply with the conditions contained in this permit or with the provisions of the Health of Animals Act and Regulations may result in the cancellation of this permit and will result in the forfeiture to the Crown of the imported thing(s) or in the removal of the thing(s) from Canada, all without compensation to, and at the expense of the importer. The importer(s) are responsible for the imported thing(s), their freedom from extraneous disease, active or latent, and genetic or other defects. The importer, his heirs, executors, successors and assigns release and discharges Her Majesty the Queen in right of Canada and the CFIA of and from all claims and demands, damages, actions or causes of action arising or to arise by reason of the importation of the thing(s) and agrees to indemnify and save harmless Her Majesty the Queen in right of Canada and the CFIA from and against all actions, damages, claims and demands which may be brought in respect of or arising out of the importation of such thing(s), any contamination with extraneous disease or other defects.



Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
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IMPORT PERMIT

PERMIS D'IMPORTATION

Page 3 of 3

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Importer/Importateur

LONDON HEALTH SCIENCES CENTRE

LONDON REGIONAL CANCER PROGRAM
790 COMMISSIONERS ROAD EAST
LONDON, ONTARIO
N6A4L6

Applicant Name: TREVOR G. SHEPHERD
Phone: 519-685-8500 EXT 56347 Fax: 519-685-8673
Email: TSHEPHE6@UWO.CA

Exporter/Exportateur

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DEPARTMENT OF MOLECULAR GENETICS & MICROBIOLOGY
GAINESVILLE, FLORIDA
UNITED

Contact: Dr. Grant McFadden / Sherin Smallwood
Phone: (352) 273-6852 Fax: (352) 273-6849

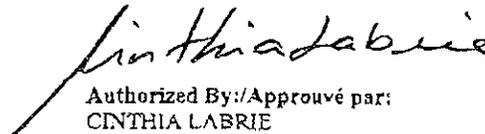
Selected Conditions / Conditions Choies (Continued/Suite)

Additional Conditions Additionnelles

MYXOMA VIRUS - PLEASE SEE ADDITIONAL CONDITIONS AT THE END OF THIS PERMIT.

(TO BE USED IN VITRO ONLY IN ROOM A4-908, CANCER RESEARCH LABORATORY, LONDON HEALTH SCIENCES CENTRE, LONDON, ON)

1. Employees must not visit farms, rabbitries or petting zoos for at least 14 days after working in the laboratory with live myxomavirus.
2. Employees working in this laboratory must not handle domestic or wild rabbits.
3. All activities with infectious materials are conducted in a biological safety cabinet.
4. Personal items such as purses and outdoor clothing must not be brought into the laboratory zone (otherwise it must be entirely decontaminated before it leaves the laboratory zone).
5. Personnel entering the laboratory zone must wear lab coats, hair nets and dedicated shoes. Contaminated clothing must be decontaminated prior to laundering.
6. Where a known or suspected aerosol exposure has occurred (e.g. dropping infectious materials) a shower is required on exit from the laboratory zone.
7. Research must not be performed on any animals without prior approval from the OBCS, CFIA.
8. This import permit must be read by personnel; employees must certify in writing that they have understood the conditions of this permit and will abide by them.


Authorized By:/Approuvé par:
CINTHIA LABRIE

For the Minister of Agriculture and Agri-Food
Pour le ministre d'agriculture et agroalimentaire



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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

- | | | | | |
|---------------|--------------------|-----------|-------------------|----------------|
| • 5K | • CIE85 | • J52 | • MC4100 (MuLac) | • U5/41 |
| • 58 | • DH1 | • J53 | • MG1655 | • W208 |
| • 58-161 | • DH10 GOLD | • JC3272 | • MM294 | • W945 |
| • 679 | • DH10B | • JC7661 | • MS101 | • W1485 |
| • 1532 | • DH5 | • JC9387 | • NC-7 | • W3104 |
| • AB284 | • DH5-alpha | • JF1504 | • Nissle 1917 | • W3110 |
| • AB311 | • DP50 | • JF1508 | • One Shot STBL3 | • WA704 |
| • AB1157 | • DY145 | • JF1509 | • OP50 | • WP2 |
| • AB1206 | • DY380 | • JJ055 | • P678 | • X1854 |
| • AG1 | • E11 | • JM83 | • PA309 | • X2160T |
| • B | • EJ183 | • JM101 | • PK-5 | • X2541 |
| • BB4 | • EL250 | • JM109 | • PMC103 | • X2547T |
| • BD792 | • EMG2 | • K12 | • PR13 | • XL1-BLUE |
| • BL21 | • EPI 300 | • KC8 | • Rri | • XL1-BLUE-MRF |
| • BL21 (DE3) | • EZ10 | • KA802 | • RV308 | • XL0LR |
| • BM25.8 | • FDA Seattle 1946 | • KAM32 | • S17-1λ -PIR | • Y10 |
| • C | • Fusion-Blue | • KAM33 | • SCS1 | • Y1090 (1090) |
| • C-1a | • H1443 | • KAM43 | • SMR10 | • YN2980 |
| • C-3000 | • HF4714 | • LE450 | • SOLR | • W3110 |
| • C25 | • HB101 | • LE451 | • SuperchargeEZ10 | • WG1 |
| • C41 (DE3) | • HS(PFAMP)R | • LE452 | • SURE | • WG439 |
| • C43 (DE3) | • Hfr3000 | • MB408 | • TOP10 | • WG443 |
| • C600 | • Hfr3000 X74 | • MBX1928 | • TG1 | • WG445 |
| • Cavalli Hfr | • HMS174 | • MC1061 | | |

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

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

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

Sincerely,

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

Info on Cell Line(s)

Cell Biology

ATCC® Number: **CRL-1573™** Price: **\$256.00**

Designations: **293 [HEK-293]**
Depositors: FL Graham
Biosafety Level: 2 [CELLS CONTAIN ADENOVIRUS]
Shipped: frozen
Medium & Serum: See Propagation
Growth Properties: adherent
Organism: *Homo sapiens* (human)
epithelial

Morphology: 

Source: **Organ:** embryonic kidney
Cell Type: transformed with adenovirus 5 DNA

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Restrictions: These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.

Applications: efficacy testing [92587]
transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
virucide testing [92579]

Receptors: vitronectin, expressed

Tumorigenic: YES
Amelogenin: X
CSF1PO: 11,12
D13S317: 12,14
D16S539: 9,13

DNA Profile (STR): D5S818: 8,9
D7S820: 11,12
THO1: 7,9.3
TPOX: 11
vWA: 16,19

Cytogenetic Analysis:

Related Links ▶

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- [community](#)

Cell Biology

ATCC® Number: **HTB-75™** [Order this Item](#) Price: **\$329.00**

Designations: **Caov-3**
 Depositors: J Fogh
 Biosafety Level: 1
 Shipped: frozen
 Medium & Serum: [See Propagation](#)
 Growth Properties: adherent
 Organism: *Homo sapiens* (human)
 epithelial

Morphology: 

Source: **Organ:** ovary
Disease: adenocarcinoma

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Restrictions: The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1976

Amelogenin: X
 CSF1PO: 10,13
 D13S317: 12
 D16S539: 9
 DNA Profile (STR): D5S818: 12
 D7S820: 10
 TH01: 7
 TPOX: 8,10
 vWA: 16,18

Isoenzymes: AK-1, 1
 ES-D, 1
 G6PD, B
 GLO-I, 1-2
 Me-2, 2
 PGM1, 1
 PGM3, 1

Related Links ▶

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BioProducts

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Cell Biology

ATCC® Number:	HTB-161™	<input type="button" value="Order this Item"/>	Price:	\$272.00
Designations:	NIHOVCAR-3			Related Links ▶
Depositors:	R Ozols, TC Hamilton			NCBI Entrez Search
<u>Biosafety Level:</u>	1			Cell Micrograph
Shipped:	frozen			Make a Deposit
Medium & Serum:	See Propagation			Frequently Asked Questions
Growth Properties:	adherent			Material Transfer Agreement
Organism:	<i>Homo sapiens</i> (human) epithelial			Technical Support
Morphology:				Related Cell Culture Products
Source:	Organ: ovary Disease: adenocarcinoma Cell Type: epithelial			Login Required ▶
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.			Product Information Sheet
Isolation:	Isolation date: 1982			BioProducts
Applications:	transfection host (Roche FuGENE® Transfection Reagents)			Cell, microbial and molecular genomics products for the life sciences
Receptors:	androgen receptor, positive; estrogen receptor, positive; progesterone receptor, positive			
Tumorigenic:	Yes			
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12 D16S539: 12 D5S818: 11,12 D7S820: 10 THO1: 9,9.3 TPOX: 8 vWA: 17			BioServices

- [sciences](#)

- [level services](#)

Cell Biology

ATCC® Number: **HTB-77™** [Order this Item](#) Price: **\$272.00**

Designations: **SK-OV-3 [SKOV-3]**

Depositors: G Trempe, LJ Old

[Biosafety Level:](#) 1

Shipped: frozen

Medium & Serum: [See Propagation](#)

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

Source: **Organ:** ovary
Disease: adenocarcinoma
Derived from metastatic site: ascites

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

Restrictions: The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.

Isolation: **Isolation date:** 1973

Applications: transfection host ([Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents](#))

Tumorigenic: Yes

Antigen Expression: Blood Type B; Rh+

DNA Profile (STR): Amelogenin: X
CSF1PO: 11
D13S317: 8,11
D16S539: 12
D5S818: 11
D7S820: 13,14
TH01: 9,9.3
TPOX: 8,11
vWA: 17,18

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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: LIBRARY EFFICIENCY DH5ALPHA COMPETENT CELLS
 Stock Number: 18263012

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
DIMETHYL SULFOXIDE	67-68-5	3 - 7

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LIBRARY EFFICIENCY DHSALPHA COMPETENT CELLS
 INVITROGEN CORPORATION
 MSDS ID: 18263

3. HAZARDS IDENTIFICATION

***** EMERGENCY OVERVIEW *****
 Warning!
 Irritant.
 Harmful if absorbed.

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.
 No toxicity expected from inhalation.

Ingestion:
 Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

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. Get medical attention if irritation develops or persists.

Inhalation:
 Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion:
 Do not induce vomiting and seek medical attention immediately. Drink two

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4. FIRST AID MEASURES (CONT.)

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:
Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trichloride, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

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:
Carbon dioxide Carbon monoxide Sulfur containing gases

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.

6. ACCIDENTAL RELEASE MEASURES (CONT.)

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 the expertise of employees in the area responding to the spill. Ventilate the contaminated area. Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

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 ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
 Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL	AGCIH TWA
DIMETHYL SULFOXIDE	(ppm) Not established.	(ppm) Not established.

Engineering Controls:

Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
 Safety glasses should be the minimum eye protection.
 Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash

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8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
 Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. 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. Gloves should be used as minimum hand protection.

Respiratory:
 Use supplied-air respiratory equipment as required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state: Liquid solution / suspension
Odor: No odor.

Not established.
 Not established.

10. STABILITY AND REACTIVITY

Stability:
 Stable under normal conditions.

Conditions to Avoid:
 Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalis. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO

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MSDS ID: 18263

10. STABILITY AND REACTIVITY (CONT.)

degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Sulfur containing gases.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG

Target Organs: Blood. Eyes. Skin.

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.
Biodegrades slowly.

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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.
 Subsidiary Hazards:

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
 DIMETHYL SULFOXIDE D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES: None assigned.
 PRODUCT SAFETY PHRASES: Not applicable.
 PRODUCT CLASSIFICATION:

MATERIAL SAFETY DATA SHEET

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15. REGULATORY INFORMATION (CONT.)

Not classified

Component DIMETHYL SULFOXIDE
 EINECS Number 200-664-3

16. OTHER INFORMATION

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

Abbreviations

- N/A - Data is not applicable or not available
- SARA - Superfund and Reauthorization Act
- HMS - 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.

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045

<http://www.vectorbiolabs.com>

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:

All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5

CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doublestranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.

HOST RANGE: Humans and animals

INCUBATION PERIOD: from 1-10 days

MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection

CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.

PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.

SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT:

Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.

Inhalation: N/A

Ingestion: Wash out mouth with water. Call a physician

Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.

Wipe up carefully.

Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.

PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:

Access to the laboratory is limited.

Work surfaces are decontaminated before and after each procedure

Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.

Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.

Laboratory coats are worn in and are removed before leaving the laboratory.

Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization

STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

Material Safety Data Sheet



Stratagene StrataClone PCR Cloning Kit, Catalog #240205

1. Product and company identification

Product name	: Stratagene StrataClone PCR Cloning Kit, Catalog #240205
Part No.	: StrataClone Vector Mix 240205-51 amp/kan StrataClone Cloning Buffer 240205-54 StrataClone Control Insert 240205-53 StrataClone SoloPack competent cells 200185-41 pUC18 Control Plasmid DNA 200231-42
Manufacturer / Supplier	: Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Emergency telephone number	: 1-800-894-1304
Use of the substance/preparation	: Chemical Kit
Validation date	: 10/01/2009

2. Hazards identification

Physical state	: StrataClone Vector Mix amp/kan Liquid. StrataClone Cloning Buffer Liquid. StrataClone Control Insert Liquid. StrataClone SoloPack competent cells Liquid. pUC18 Control Plasmid DNA Liquid.
OSHA/HCS status	: StrataClone Vector Mix amp/kan This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). StrataClone Cloning Buffer This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). StrataClone Control Insert While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product. StrataClone SoloPack competent cells This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200). pUC18 Control Plasmid DNA While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.

2. Hazards identification

Emergency overview- Label Statement	: StrataClone Vector Mix amp/kan	MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
	StrataClone Cloning Buffer	CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
	StrataClone Control Insert	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
	StrataClone SoloPack competent cells	MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
	pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
	StrataClone Vector Mix amp/kan	Slightly irritating to the eyes, skin and respiratory system. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Avoid contact with eyes. Avoid prolonged or repeated contact with skin. Contains material that may cause target organ damage, based on animal data. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.
	StrataClone Cloning Buffer	Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Contains material that may cause target organ damage, based on animal data.
	StrataClone Control Insert	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	StrataClone SoloPack competent cells	Slightly irritating to the eyes, skin and respiratory system. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Avoid contact with eyes. Avoid prolonged or repeated contact with skin. Contains material that may cause target organ damage, based on animal data. Use only with adequate ventilation. Keep container tightly closed and sealed until ready for use. Wash thoroughly after handling.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	StrataClone Vector Mix amp/kan	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	StrataClone Cloning Buffer	Contains material which may cause damage to the following organs: skin, stomach.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	pUC18 Control Plasmid DNA	Not available.
	Routes of entry	: StrataClone Vector Mix amp/kan
StrataClone Cloning Buffer		Ingestion.
StrataClone Control Insert		Not applicable.
StrataClone SoloPack competent cells		Not applicable.
pUC18 Control Plasmid DNA		Not applicable.

Potential acute health effects

2. Hazards identification

Eyes	: StrataClone Vector Mix amp/kan	Slightly irritating to the eyes.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	Slightly irritating to the eyes.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Skin	: StrataClone Vector Mix amp/kan	Slightly irritating to the skin.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	Slightly irritating to the skin.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Inhalation	: StrataClone Vector Mix amp/kan	Slightly irritating to the respiratory system.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	Slightly irritating to the respiratory system.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Ingestion	: StrataClone Vector Mix amp/kan	No known significant effects or critical hazards.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	No known significant effects or critical hazards.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Medical conditions aggravated by over-exposure	: StrataClone Vector Mix amp/kan	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged exposure to the substance can produce lung damage. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to the substance can produce target organs damage.
	StrataClone Cloning Buffer	Repeated or prolonged exposure to the substance can produce target organs damage.
	StrataClone Control Insert	Not applicable.
	StrataClone SoloPack competent cells	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged exposure to the substance can produce lung damage. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation. Repeated or prolonged exposure to the substance can produce target organs damage.
	pUC18 Control Plasmid DNA	Not applicable.

2. Hazards identification

Over-exposure signs/symptoms	: StrataClone Vector Mix amp/kan	Not applicable.
	StrataClone Cloning Buffer	Not applicable.
	StrataClone Control Insert	Not applicable.
	StrataClone SoloPack competent cells	Not applicable.
	pUC18 Control Plasmid DNA	Not applicable.

See toxicological information (section 11)

3. Composition/information on ingredients

<u>Name</u>	<u>CAS number</u>	<u>%</u>
StrataClone Vector Mix amp/kan		
Glycerol	56-81-5	30 - 60
StrataClone Cloning Buffer		
Polyethylene glycol	25322-68-3	10 - 30
Sodium chloride	7647-14-5	1 - 5
StrataClone SoloPack competent cells		
Glycerol	56-81-5	10 - 30

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4. First aid measures

Eye contact	: StrataClone Vector Mix amp/kan	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
	StrataClone Cloning Buffer	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
	StrataClone Control Insert	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
	StrataClone SoloPack competent cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
	pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	: StrataClone Vector Mix amp/kan	Wash with soap and water. Get medical attention if adverse health effects persist or are severe.
	StrataClone Cloning Buffer	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
	StrataClone Control Insert	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
	StrataClone SoloPack competent cells	Wash with soap and water. Get medical attention if adverse health effects persist or are severe.
	pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash

4. First aid measures

		clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	: StrataClone Vector Mix amp/kan	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if adverse health effects persist or are severe.
	StrataClone Cloning Buffer	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
	StrataClone Control Insert	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
	StrataClone SoloPack competent cells	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention if adverse health effects persist or are severe.
	pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
Ingestion	: StrataClone Vector Mix amp/kan	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
	StrataClone Cloning Buffer	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
	StrataClone Control Insert	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
	StrataClone SoloPack competent cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
	pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	: StrataClone Vector Mix amp/kan	Not applicable.
	StrataClone Cloning Buffer	Not applicable.
	StrataClone Control Insert	Not applicable.
	StrataClone SoloPack competent cells	Not applicable.
	pUC18 Control Plasmid DNA	Not applicable.
Notes to physician	: No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5 . Fire-fighting measures

Flammability of the product	: StrataClone Vector Mix amp/kan StrataClone Cloning Buffer StrataClone Control Insert StrataClone SoloPack competent cells pUC18 Control Plasmid DNA	Non-flammable. Non-flammable. Non-flammable. Non-flammable. Non-flammable.
Products of combustion	: StrataClone Vector Mix amp/kan StrataClone Cloning Buffer StrataClone Control Insert StrataClone SoloPack competent cells pUC18 Control Plasmid DNA	Decomposition products may include the following materials: carbon oxides Decomposition products may include the following materials: carbon oxides halogenated compounds metal oxide/oxides No specific data. Decomposition products may include the following materials: carbon oxides halogenated compounds metal oxide/oxides No specific data.
<u>Extinguishing media</u>		
Suitable	: StrataClone Vector Mix amp/kan StrataClone Cloning Buffer StrataClone Control Insert StrataClone SoloPack competent cells pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire. Use an extinguishing agent suitable for the surrounding fire.
Not suitable	: StrataClone Vector Mix amp/kan StrataClone Cloning Buffer StrataClone Control Insert StrataClone SoloPack competent cells pUC18 Control Plasmid DNA	Not applicable. Not applicable. Not applicable. Not applicable. Not applicable.
Special protective equipment for fire-fighters	: Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	

6 . Accidental release measures

Personal precautions	: StrataClone Vector Mix amp/kan StrataClone Cloning Buffer	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8). No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
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6 . Accidental release measures

	StrataClone Control Insert	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
	StrataClone SoloPack competent cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
	pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	: StrataClone Vector Mix amp/kan	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
	StrataClone Cloning Buffer	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
	StrataClone Control Insert	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
	StrataClone SoloPack competent cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
	pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up		
Small spill	: StrataClone Vector Mix amp/kan	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
	StrataClone Cloning Buffer	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
	StrataClone Control Insert	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
	StrataClone SoloPack competent cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container.

6 . Accidental release measures

pUC18 Control Plasmid
DNA

disposal container. Dispose of via a licensed waste disposal contractor.

Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling

- : StrataClone Vector Mix amp/kan Wash thoroughly after handling.
- StrataClone Cloning Buffer Wash thoroughly after handling.
- StrataClone Control Insert Wash thoroughly after handling.
- StrataClone SoloPack competent cells Wash thoroughly after handling.
- pUC18 Control Plasmid DNA Wash thoroughly after handling.

Storage

- : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8 . Exposure controls/personal protection

Product name

Exposure limits

United States

StrataClone Vector Mix amp/kan
Glycerol

ACGIH TLV (United States, 1/2008).

TWA: 10 mg/m³ 8 hour(s). Form: Mist

OSHA PEL (United States, 11/2006).

TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction

TWA: 15 mg/m³ 8 hour(s). Form: Total dust

OSHA PEL 1989 (United States, 3/1989).

TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction

TWA: 10 mg/m³ 8 hour(s). Form: Total dust

StrataClone Cloning Buffer

Polyethylene glycol

AIHA WEEL (United States, 1/2008).

TWA: 10 mg/m³ 8 hour(s). Form: Aerosol

StrataClone SoloPack competent cells

Glycerol

ACGIH TLV (United States, 1/2008).

TWA: 10 mg/m³ 8 hour(s). Form: Mist

OSHA PEL (United States, 11/2006).

TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction

TWA: 15 mg/m³ 8 hour(s). Form: Total dust

OSHA PEL 1989 (United States, 3/1989).

TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction

TWA: 10 mg/m³ 8 hour(s). Form: Total dust

Consult local authorities for acceptable exposure limits.

Engineering measures

- : Use only with adequate ventilation. If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

8 . Exposure controls/personal protection

Eyes	: Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.
Skin	: Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.
Respiratory	: Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Hands	: Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Other protection	: Not available.
Hygiene measures	: Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	: StrataClone Vector Mix amp/kan	Liquid.
	StrataClone Cloning Buffer	Liquid.
	StrataClone Control Insert	Liquid.
	StrataClone SoloPack competent cells	Liquid.
	pUC18 Control Plasmid DNA	Liquid.
pH	: StrataClone Vector Mix amp/kan	Not available.
	StrataClone Cloning Buffer	Not available.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Not available.
	pUC18 Control Plasmid DNA	8.2 [Basic.]
Boiling/condensation point	: StrataClone Vector Mix amp/kan	Lowest known value: 100°C (212°F) (Water). Weighted average: 195°C (383°F)
	StrataClone Cloning Buffer	Lowest known value: 100°C (212°F) (Water).
	StrataClone Control Insert	Lowest known value: 100°C (212°F) (Water).
	StrataClone SoloPack competent cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 138°C (280.4°F)
	pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
Melting/freezing point	: StrataClone Vector Mix amp/kan	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 9.9°C (49.8°F)
	StrataClone Cloning Buffer	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	StrataClone Control Insert	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
	StrataClone SoloPack competent cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient:

9 . Physical and chemical properties

		Glycerol. Weighted average: 3.96°C (39.1°F)
	pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
Vapor density	: StrataClone Vector Mix amp/kan	Highest known value: 3.1 (Air = 1) (Glycerol).
	StrataClone Cloning Buffer	Not available.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Highest known value: 3.1 (Air = 1) (Glycerol).
	pUC18 Control Plasmid DNA	Not available.
Solubility	: StrataClone Vector Mix amp/kan	Easily soluble in the following materials: cold water and hot water.
	StrataClone Cloning Buffer	Easily soluble in the following materials: cold water and hot water.
	StrataClone Control Insert	Easily soluble in the following materials: cold water and hot water.
	StrataClone SoloPack competent cells	Not available.
	pUC18 Control Plasmid DNA	Easily soluble in the following materials: cold water and hot water.

10 . Stability and reactivity

Stability and reactivity	: The product is stable. Under normal conditions of storage and use, hazardous polymerization will not occur.
Incompatibility with various substances	: Highly reactive or incompatible with the following materials: oxidizing materials. Reactive or incompatible with the following materials: reducing materials, metals, acids, alkalis and moisture. Slightly reactive or incompatible with the following materials: organic materials.
Hazardous decomposition products	: StrataClone Vector Mix amp/kan Under normal conditions of storage and use, hazardous decomposition products should not be produced. StrataClone Cloning Buffer Under normal conditions of storage and use, hazardous decomposition products should not be produced. StrataClone Control Insert Under normal conditions of storage and use, hazardous decomposition products should not be produced. StrataClone SoloPack competent cells Under normal conditions of storage and use, hazardous decomposition products should not be produced. pUC18 Control Plasmid DNA Under normal conditions of storage and use, hazardous decomposition products should not be produced.

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Glycerol	LD50 Oral	Rat	12600 mg/kg	-
Eyes	: StrataClone Vector Mix amp/kan	Slightly irritating to the eyes.		
	StrataClone Cloning Buffer	No known significant effects or critical hazards.		
	StrataClone Control Insert	No known significant effects or critical hazards.		
	StrataClone SoloPack competent cells	Slightly irritating to the eyes.		
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.		

11 . Toxicological information

Skin	: StrataClone Vector Mix amp/kan	Slightly irritating to the skin.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	Slightly irritating to the skin.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Inhalation	: StrataClone Vector Mix amp/kan	Slightly irritating to the respiratory system.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	Slightly irritating to the respiratory system.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
Ingestion	: StrataClone Vector Mix amp/kan	No known significant effects or critical hazards.
	StrataClone Cloning Buffer	No known significant effects or critical hazards.
	StrataClone Control Insert	No known significant effects or critical hazards.
	StrataClone SoloPack competent cells	No known significant effects or critical hazards.
	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.

Potential chronic health effects

Chronic effects	: Contains material that may cause target organ damage, based on animal data.
Carcinogenicity	: No known significant effects or critical hazards.
Mutagenicity	: No known significant effects or critical hazards.
Teratogenicity	: No known significant effects or critical hazards.
Developmental effects	: No known significant effects or critical hazards.
Fertility effects	: No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation	: Adverse symptoms may include the following: respiratory tract irritation coughing	
Ingestion	: No specific data.	
Skin	: Adverse symptoms may include the following: irritation redness	
Eyes	: Adverse symptoms may include the following: irritation watering redness	
Target organs	: StrataClone Vector Mix amp/kan	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	StrataClone Cloning Buffer	Contains material which may cause damage to the following organs: skin, stomach.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	pUC18 Control Plasmid	Not available.

11 . Toxicological information

	DNA	
Other adverse effects	: StrataClone Vector Mix amp/kan	Not available.
	StrataClone Cloning Buffer	Not available.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Not available.
	pUC18 Control Plasmid DNA	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Polyethylene glycol	-	Acute LC50 >1000000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification	: StrataClone Vector Mix amp/kan	Target organ effects
	StrataClone Cloning Buffer	Target organ effects
	StrataClone Control Insert	Not regulated.
	StrataClone SoloPack competent cells	Target organ effects
	pUC18 Control Plasmid DNA	Not regulated.

15 . Regulatory information

	StrataClone Vector Mix amp/kan	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	StrataClone Cloning Buffer	Contains material which may cause damage to the following organs: skin, stomach.
	StrataClone Control Insert	Not available.
	StrataClone SoloPack competent cells	Contains material which may cause damage to the following organs: kidneys, upper respiratory tract, skin, eye, lens or cornea.
	pUC18 Control Plasmid DNA	Not available.
U.S. Federal regulations	: StrataClone Vector Mix amp/kan	TSCA 8(a) PAIR: Poly(oxy-1,2-ethanediy), .alpha.-[4-(1,1,3,3-tetramethylbutyl)phenyl]-.omega.-hydroxy- United States inventory (TSCA 8b) : All components are listed or exempted.
	StrataClone Cloning Buffer	United States inventory (TSCA 8b) : All components are listed or exempted.
	StrataClone Control Insert	United States inventory (TSCA 8b) : All components are listed or exempted.
	StrataClone SoloPack competent cells	United States inventory (TSCA 8b) : All components are listed or exempted.
	pUC18 Control Plasmid DNA	United States inventory (TSCA 8b) : All components are listed or exempted.
	StrataClone Vector Mix amp/kan	SARA 302/304/311/312 extremely hazardous substances : No products were found. SARA 302/304 emergency planning and notification : No products were found. SARA 302/304/311/312 hazardous chemicals : Glycerol SARA 311/312 MSDS distribution - chemical inventory - hazard identification : Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard
	StrataClone Cloning Buffer	SARA 302/304/311/312 extremely hazardous substances : No products were found. SARA 302/304 emergency planning and notification : No products were found. SARA 302/304/311/312 hazardous chemicals : Sodium chloride SARA 311/312 MSDS distribution - chemical inventory - hazard identification : Sodium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard
	StrataClone Control Insert	SARA 302/304/311/312 extremely hazardous substances : No products were found. SARA 302/304 emergency planning and notification : No products were found. SARA 302/304/311/312 hazardous chemicals : No products were found. SARA 311/312 MSDS distribution - chemical inventory - hazard identification : No products were found.
	StrataClone SoloPack competent cells	SARA 302/304/311/312 extremely hazardous substances : No products were found. SARA 302/304 emergency planning and notification : No products were found. SARA 302/304/311/312 hazardous chemicals : Glycerol; Rubidium chloride SARA 311/312 MSDS distribution - chemical inventory - hazard identification : Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Rubidium chloride: Delayed (chronic) health hazard
	pUC18 Control Plasmid	SARA 302/304/311/312 extremely hazardous substances :

15 . Regulatory information

DNA	No products were found. SARA 302/304 emergency planning and notification: No products were found. SARA 302/304/311/312 hazardous chemicals: No products were found. SARA 311/312 MSDS distribution - chemical inventory - hazard identification: No products were found.
StrataClone Vector Mix amp/kan	Clean Water Act (CWA) 307: No products were found.
StrataClone Cloning Buffer	Clean Water Act (CWA) 307: No products were found.
StrataClone Control Insert	Clean Water Act (CWA) 307: No products were found.
StrataClone SoloPack competent cells	Clean Water Act (CWA) 307: No products were found.
pUC18 Control Plasmid DNA	Clean Water Act (CWA) 307: No products were found.
StrataClone Vector Mix amp/kan	Clean Water Act (CWA) 311: Edetic acid
StrataClone Cloning Buffer	Clean Water Act (CWA) 311: No products were found.
StrataClone Control Insert	Clean Water Act (CWA) 311: Edetic acid
StrataClone SoloPack competent cells	Clean Water Act (CWA) 311: Potassium hydroxide
pUC18 Control Plasmid DNA	Clean Water Act (CWA) 311: Edetic acid
StrataClone Vector Mix amp/kan	Clean Air Act (CAA) 112 accidental release prevention: No products were found.
StrataClone Cloning Buffer	Clean Air Act (CAA) 112 accidental release prevention: No products were found.
StrataClone Control Insert	Clean Air Act (CAA) 112 accidental release prevention: No products were found.
StrataClone SoloPack competent cells	Clean Air Act (CAA) 112 accidental release prevention: No products were found.
pUC18 Control Plasmid DNA	Clean Air Act (CAA) 112 accidental release prevention: No products were found.
StrataClone Vector Mix amp/kan	Clean Air Act (CAA) 112 regulated flammable substances: No products were found.
StrataClone Cloning Buffer	Clean Air Act (CAA) 112 regulated flammable substances: No products were found.
StrataClone Control Insert	Clean Air Act (CAA) 112 regulated flammable substances: No products were found.
StrataClone SoloPack competent cells	Clean Air Act (CAA) 112 regulated flammable substances: No products were found.
pUC18 Control Plasmid DNA	Clean Air Act (CAA) 112 regulated flammable substances: No products were found.
StrataClone Vector Mix amp/kan	Clean Air Act (CAA) 112 regulated toxic substances: No products were found.
StrataClone Cloning Buffer	Clean Air Act (CAA) 112 regulated toxic substances: No products were found.
StrataClone Control Insert	Clean Air Act (CAA) 112 regulated toxic substances: No products were found.
StrataClone SoloPack competent cells	Clean Air Act (CAA) 112 regulated toxic substances: No products were found.
pUC18 Control Plasmid DNA	Clean Air Act (CAA) 112 regulated toxic substances: No products were found.

15 . Regulatory information

State regulations

: StrataClone Vector Mix
amp/kan

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol
Rhode Island Hazardous Substances: None of the components are listed.

StrataClone Cloning
Buffer

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the components are listed.
Rhode Island Hazardous Substances: None of the

15 . Regulatory information

components are listed.

StrataClone Control Insert

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

StrataClone SoloPack competent cells

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following

15 . Regulatory information

pUC18 Control Plasmid
DNA

components are listed: Glycerol

Rhode Island Hazardous Substances: None of the components are listed.

Connecticut Carcinogen Reporting: None of the components are listed.

Connecticut Hazardous Material Survey: None of the components are listed.

Florida substances: None of the components are listed.

Illinois Chemical Safety Act: None of the components are listed.

Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.

Louisiana Reporting: None of the components are listed.

Louisiana Spill: None of the components are listed.

Massachusetts Spill: None of the components are listed.

Massachusetts Substances: None of the components are listed.

Michigan Critical Material: None of the components are listed.

Minnesota Hazardous Substances: None of the components are listed.

New Jersey Hazardous Substances: None of the components are listed.

New Jersey Spill: None of the components are listed.

New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.

New York Acutely Hazardous Substances: None of the components are listed.

New York Toxic Chemical Release Reporting: None of the components are listed.

Pennsylvania RTK Hazardous Substances: None of the components are listed.

Rhode Island Hazardous Substances: None of the components are listed.

State regulations -
California Prop. 65

: No products were found.

16 . Other information

Label requirements

: StrataClone Vector Mix
amp/kan

MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

StrataClone Cloning
Buffer

StrataClone Control Insert

NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

StrataClone SoloPack
competent cells

MAY CAUSE RESPIRATORY TRACT, EYE AND SKIN IRRITATION. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

pUC18 Control Plasmid
DNA

NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.

Date of issue

: 10/01/2009

Version

: 1

[Notice to reader](#)

16 . Other information

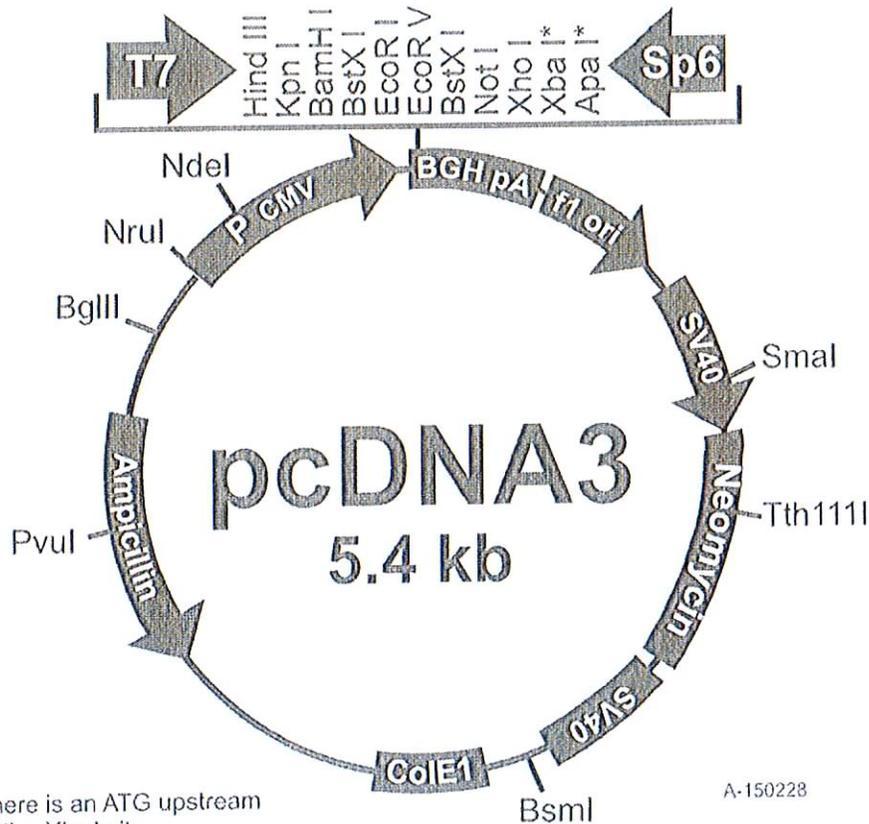
DISCLAIMER: This Material Safety Data Sheet is offered without charge to the clients of Agilent Technologies. Data is the most current available to Agilent Technologies at the time of preparation and is issued as a matter of information only, no warranty as to its accuracy or completeness is expressed or implied.

Comments for pcDNA3:
5446 nucleotides



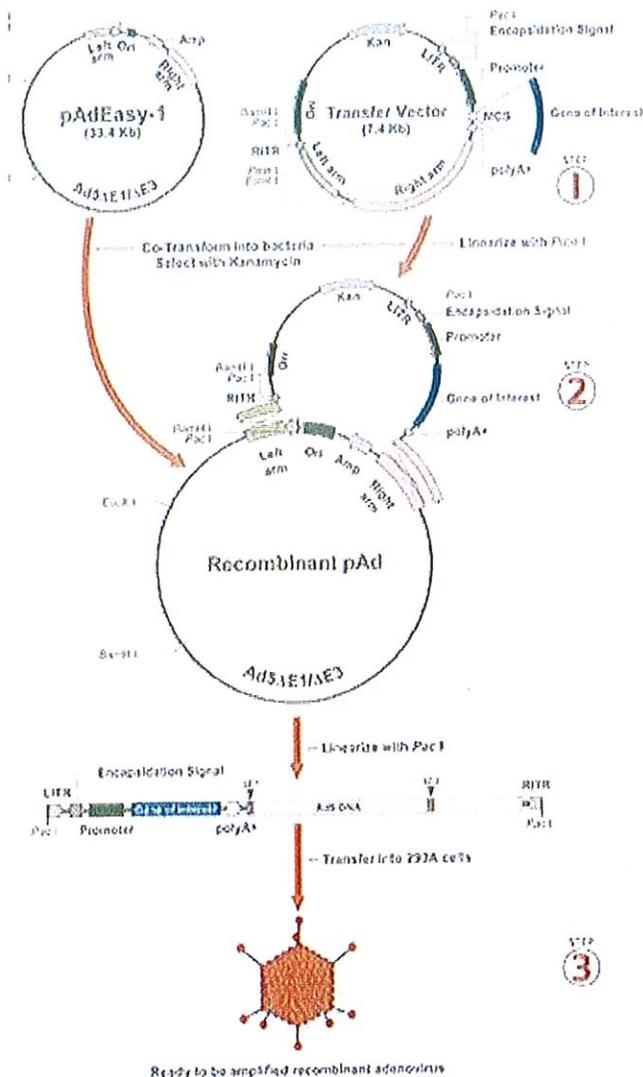
CMV promoter: bases 209-863
T7 promoter: bases 864-882
Polylinker: bases 889-994
Sp6 promoter: bases 999-1016
BGH poly A: bases 1018-1249
SV40 promoter: bases 1790-2115
SV40 origin of replication: bases 1984-2069
Neomycin ORF: bases 2151-2945
SV40 poly A: bases 3000-3372
ColE1 origin: bases 3632-4305
Ampicillin ORF: bases 4450-5310

Section 4



The sequence of pcDNA3 has been compiled from information in sequence databases, published sequences, and other sources. This vector has not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department.

Adenovirus Using AdEasy™



AdEasy™

- **Save weeks of time by avoiding multiple plaque assay steps**
- **Exploit the robust, efficient E.Coli homologous recombination system**
- **Comprehensive kit components including 293 cells and bacterial cells**

Reference Library

- [Protocol](#)
- [Sequences](#)
- [Plasmid Maps](#)
- [Product Profile](#)
- [FAQ](#)

The AdEasy™ system is used to rapidly generate recombinant adenovirus without the need for time consuming plaque purification. Developed by T.C He et al (12), the AdEasy™ system exploits E. coli's robust, efficient recombination machinery thereby avoiding restriction-ligation involving the unwieldy (36kb) adenovirus genome. Bacterial and 293 cells that are essential to the technique are included in every kit with each kit providing enough reagents to generate up to 5 recombinant adenoviruses. The highly detailed user manual is an invaluable resource for beginners as well as those experienced in this field of work.

STEP 1 : The cDNA of interest is first cloned into a transfer vector.

STEP 2 : The resulting plasmid is linearized with Pme I and co-transformed into E. coli strain BJ5183 together with pAdEasy-1, the viral DNA plasmid. Recombinants are selected with kanamycin and screened by restriction enzyme analysis.

STEP 3 : The recombinant adenoviral construct is then cleaved with Pac I to expose its ITR (Inverted Terminal Repeats) and transfected into QBI-293A cells to produce viral particles.

AdEasy™ Kit

The AdEasy™ kit comes in a complete package format containing all the principal components and controls for the construction of 5 recombinant viruses. Each AdEasy™ system kit includes all the components listed below plus your choice of one transfer vector.

Cat No.	Product	Transfer Vector	Quantity
AES1000	AdEasy™ basic kit	without transfer vector	5 assays
AES1000A	AdEasy™ kit	pShuttle (AES1020)	5 assays
AES1000B	AdEasy™ kit	pShuttle-CMV (AES1021)	5 assays

Description of Plasmids and Kit Reagents

Contents of the AdEasy™ Kit

Cat No.	Product	Description	Quantity	Storage
AES1010	pAdEasy-1 ccc DNA plasmid	Ad5.ΔE1/ΔE3	0.5µg (100ng/µl; 5µl)	-20°C
AES1005	BJ5183 EC Electrocompetent cells	BJ5183	5 x 80µl	-80°C
AES1007K	DH5α EC Electrocompetent cells	DH5α	5 x 40µl	-80°C
AES0503	QBI-293A cells	Frozen 293 cell line	1ml (1 X 10 ⁵ cells/ml)	-150°C
	QBI-Infect Ad5.CMV-LacZΔE1/ΔE3	Viral particles of (in complete DMEM)	1ml (>1000 PFU/ml)	-80°C
	CaCl ₂ 2M	Transfection reagent	0.5ml	-20°C
	TE 0.1x	Transfection reagent	0.5ml	-20°C
	HBS 2x	Transfection reagent	3 x 1ml	-20°C
	(see above)	Choice of transfer vector	25µg (500ng/ml; 50µl)	-20°C

Storage
-20°C to -150°C