**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOLOGICAL AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: October 14, 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 | Fred Possmayer |
| DEPARTMENT | OBS/GYN and BIOCHEM |
| ADDRESS | DSB5009 |
| PHONE NUMBER | 519 6612111, E80972, E80971, EE83528 T Regnault, E84823 D Hardy |
| EMERGENCY PHONE NUMBER(S) | 519 438-0773 (home) |
| EMAIL | fpossmay@uwo.ca |

Location of experimental work to be carried out: Building(s) Dental Sciences Building
Room(s) DSB2019A, DSB5004, DSB5006

*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: CIHR
GRANT TITLE(S): Lipid-lipid and lipid-protein interactions in pulmonary surfactant

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

| Name | UWO E-mail Address | Date of Biosafety Training |
|---|---|---|
| Lin Zhao | Lzhoa3@uwo.ca | Needs retraining |
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**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.**

Culture experiments will be conducted in Laboratory DSB2019 which is shared with Drs, Dean Betts, Tim Regnault and Dan Hardy. Experiments are conducted in accordance with UWO's Laboratory Health and Safety Manual. In general, experiments are conducted by my research associate, Dr. Lin Zhao, whom I share with Drs. T. Regnault and D. Hardy.

Our cell lines are stored in a liquid nitrogen depository in the laboratory of Dr. Moshmi Bhattacharya. Culture manipulations are conducted, where possible, in an approved biosafety cabinet in laboratory DSB 5019. This facility is approved and has been inspected recently.

After studies, the cultures, samples and any other potentially contaminated materials are inactivated, for example with isopropanol where appropriate, bagged in Biohazard disposable containers and autoclaved prior to disposal.

In general, standard commercial vectors and reagents are employed for genetic manipulation of RNA and DNA. One change over our previous protocols is that we propose to introduce the use of shRNAi to downregulate expression of the three known Lipid Phosphate Phosphohydrolase isoforms (Nanjundan M and Possmayer F., 2003, Pulmonary phosphatidic acid phosphatase and lipid phosphate phosphohydrolase. *Am J Physiol Lung Cell Mol Physiol* 284: L1-23). These are available commercially as lentiviral vector constructs. At present there is no reason to suspect these materials will constitute a biohazard.

These shRNAi reagents will be purchased from OpenBiosystems and stored as glycerol stocks in a freezer.

Cholera toxin and pertussis toxin are stored lyophilized and small aqueous stocks are stored frozen.

**Please include a one page research summary or teaching protocol.**

Our laboratory's studies have been primarily related to various aspects of pulmonary surfactant, a material which is essential for normal breathing. As part of these studies, we have been studying alveolar epithelial Type II cell lines. Type II cells, which cover <5% of the alveolar surface, synthesize and secrete pulmonary surfactant. During these studies, we examined lung epithelial $T_7$ cells, a purported Type II cell line derived from mice bearing a temperature-sensitive (TS) Large T antigen (de Mello et al. (2000)) *In Vitro Cell. Dev. Biol.* 36: 374-382). $T_7$ cells grow well at 37°C but stop dividing and differentiate at 41°C. During our studies, we discovered that this $T_7$ line had changed its phenotype from Type II to Type I. Type I cells are extremely difficult to isolate from the lung and in fact, this has only been reported a few times and by a single group. Although the number of Type I cell and Type II cells is similar, Type I cells cover >95% of the alveolar surface. These cells function in gas exchange and transport, but their functions are not well understood.

This discovery led us to examine the circumstances involved in establishing and manipulating Type I cell characteristics. In particular, we have found that cAMP affects the levels of lipid phosphate phosphohydrolase (LPP) activity and LPP isoform expression. We are currently attempting to determine the relative contributions of these isoforms to Type I cell LPP activity and the manner in which expression of these LPP isoforms and their activity is controlled. For these studies, we propose to transient infect cells with lentiviral pGFZ and pLKO.I vectors to produce shRNAi to modify LPP isoform levels. These studies will provide information on the factors regulating the alveolar Type 1 cell phenotype and the manner in which LPP isoform expression is controlled. It will also establish a new and convenient platrform for studying Type 1 cell function.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents? xO YES O 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? O YES xO NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

| Name of Biological Agent(s)* (Be specific) | Is it known to be a human pathogen? YES/NO | Is it known to be an animal pathogen? YES/NO | Is it known to be a zoonotic agent? YES/NO | Maximum quantity to be cultured at one time? (in Litres) | Source/ Supplier | PHAC or CFIA Containment Level |
|---|---|---|---|---|---|---|
| E. coli (dH5alpha) | O Yes xO No | O Yes xO No | O Yes xO No | 1-2 liters | Commercial or In house | xO 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O 1 O 2 O 2+ O 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? xO YES O 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 | O Yes O No | | |
| Rodent | O Yes O No | | |
| Non-human primate | O Yes O No | | |

| | | | |
|---|---|---|---|
| Other (specify) | O Yes O No | | |

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

| Cell Type | Is this cell type used in your work? | Specific cell line(s)* | Containment Level of each cell line | Supplier / Source of cell line(s) |
|---|---|---|---|---|
| Human | xO Yes O No | A549,H441 (lung epithelial) HEK 293(E1A oncogene) | 1 or 2? | ATCC,In house, (SEE NOTE at end). |
| Rodent | xO Yes O No | MLE-12, $T_7$ (mouse lung epithelial, SV40 large T antigen) | 2? | Jeff Whitsett,Cincinatti who developed this cell line. It is now available from ATCC Martin Post, Paediatrics, H Sick Children who obtained them from Dr Daphe de Mello who established the line. (See Notes) |
| Non-human primate | O Yes xO No | | | |
| Other (specify) | xO Yes O No | CHO ( Chinese Hamster Ovary) | | |

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

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 xO 2 O 2+ O 3

**3.0 Use of Human Source Materials**

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

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

| **Human Source Material** | **Source/Supplier /Company Name** | **Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN** | **Name of Infectious Agent (If applicable)** | **PHAC or CFIA Containment Level (Select one)** |
|---|---|---|---|---|
| Human Blood (whole) or other Body Fluid | | O Yes O Unknown | | O 1 O 2 O 2+ O 3 |
| Human Blood (fraction) or other Body Fluid | | O Yes O Unknown | | O 1 O 2 O 2+ O 3 |
| Human Organs or Tissues (unpreserved) | | O Yes O Unknown | | O 1 O 2 O 2+ O 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? xO YES O NO If no, please proceed to Section 5.0

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

| Bacteria Used for Cloning * | Plasmid(s) ** | Source of Plasmid | Gene Transfected | Describe the change that results from transformation or tranfection |
|---|---|---|---|---|
| Ecoli (dH5 alpha) | | *In house*<br><br>*In past we used bluescript to clone LPP1-3 from rat*<br><br>*We are purchasing the mouse clones in* Vector pCMV-SPORT6 *OpenBioSystems* | *LPP1-3* | *.no changes noted in past Some clones may have grown slowly* |

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
xO YES, complete table below O NO

| Virus Used for Vector Construction | Vector(s) * | Source of Vector | Gene(s) Transduced | Describe the change that results from transduction |
|---|---|---|---|---|
| *Not applicable,( we will not use viruses)* | pGPZ<br>pKIO.1 | OpenBiosystems<br>OpenBiosystems<br>" | Mouse LPP1-3<br>shRNAi | Unknown<br>Hope specific isoforms will be downregulated |

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ xO NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ xO NO
- SV 40 Large T antigen xO YES O NO
- E1A oncogene xO YES O NO
- These are present in some of the cell lines used, but will not be manipulated.
- **Other** Known oncogenes O YES, please specify _____ xO NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ x O NO

4.5 Will virus be replication defective? xO YES O NO

4.6 Will virus be infectious to humans or animals? O YES xO NO

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

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted involving a biological agent? O YES xO 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? O YES O 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? O YES, number: _____ O NO O PENDING

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

6.5 Will the agent(s) be shed by the animal: O YES O 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)? O YES xO No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES xO No

7.3 If yes, please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____O NO
- Non-human primates O YES, please specify species _____O NO
- Wild caught animals O YES, please specify species & colony # __ _____x O NO
- Birds O YES, please specify species _____O NO
- Others (wild or domestic) O YES, please specify ___We may also obtain lungs from investigators using Xenopus __laevis (e.g. Tom Drysdale, VRL, UWO) to extract surfactant_____O NO

7.4 If no live animals are used, please specify the source of the specimens: See above____ In some cases we may examine extracts from the 13-stripped ground squirrel. These extracts are from a collaboration with Dr. Jim Staples BIOLOGY and Ruud Veldhuizen Lawson Research Institute,UWO.__Dr Staples has established a colony but some of the samples may be from trapped animals._____

**8.0 Biological Toxins**

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

8.2 If YES, please name the toxin(s)__Cholera toxin, pertussis toxin_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.
Could not find a Materials Sheet

8.3 What is the $LD_{50}$ (specify species) of the toxin_____Cholera toxin 250 ug for mice. Pertussis toxin 15 ug/kg presummably for mice.

8.4 See: http://www.ehs.ufl.edu/bio/toxin.htm

8.4 How much of the toxin is handled at one time*? ____Cholera__10 ug____,Pertussis 5 ug_____

8.5 How much of the toxin is stored*? ___Cholera_____500 ug____Perussis 50 ug_____

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

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf
**Web site does not seem to function**

**9.0 Insects**

9.1 Do you use insects? O YES xO 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? O Initiate and maintain colony, give location: _____
O "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? O YES O NO
If YES Please attach the CFIA permit & describe any CFIA permit conditions

## 10.0 Plants

10.1 Do you use plants? O YES xO 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? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O 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? O YES O 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? O YES please give country of origin _____ xO N
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NC

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NC

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 atte 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 an 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. O 1 xO 2 ?O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
xO YES, date of most recent biosafety inspection: __Nov 2010_____
O NO, please certify
O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): BIO-LHRI-0020_____

**14.0 Procedures to be Followed**

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

14.1 Level 2 is considered sufficient for use of the sh RNAi vectors and the cell lines.

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

The following emergency response procedures shall be followed when a worker has been potentially exposed to a biohazardous agents via a needlestick, cut, animal bite or scratch, via mucous membrane contact, or via non-intact skin contact.

**Worker**

1. The exposed site must be washed immediately.
a) In case of a needlestick, cut, animal bite or scratch, wash with soap and water after allowing the wound to bleed freely.
b) If mucous (eyes, nose, mouth) membrane or non-intact (cuts, rash, eczema or dermatitis) skin contact, flush with water at the nearest faucet or eye wash station for a minimum of ten minutes.
2. The worker must immediately inform the Supervisor/Principal Investigator of the exposure incident.
3. The worker must seek prompt medical attention at Workplace Health (during the hours of operation), the nearest hospital emergency department or emergency clinic, or a Medical Practitioner of their choosing. Any information including the Material Safety Data Sheet or equivalent for the biohazardous agent must also be taken to the care provider.
4. The worker must provide information for a Accident/Incident Report (obtained from her/his Supervisor/Principal Investigator), describing the incident in detail, including the route of exposure and the emergency actions taken, and a description of the worker's duties as they relate to the exposure incident.

**Supervisor / Principal Investigator**

1. Supervisors/Principal Investigators must complete and sign the University Accident/Incident Report.
2. The supervisor must ensure that exposure incidents are reported within 24 hours to Human Resources, fax (519) 661-2079. The form can be found at: http://www.uwo.ca/humanresources/facultystaff/h_and_s/acc_inc/accident_inc_index.htm
3. The supervisor must refer the affected worker(s) to the nearest hospital emergency department or emergency clinic, or preferably, to Workplace Health

during hours of operation _____

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

SIGNATURE _____ Date _____

**15.0 Approvals**

1) UWO Biohazards Subcommittee SIGNATURE _____
Date _____

2) Safety Officer for the University of Western Ontario
SIGNATURE _____
Date _____

3) Safety Officer for Institution where experiments will take place (if not UWO)
SIGNATURE _____
Date _____

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

Special Conditions of Approval



dur

ing hours of
operation._____

_____

**15.0 Approvals**

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

2) Safety Officer for the University of Western Ontario
SIGNATURE:_____
Date:_____

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

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

Special Conditions of Approval:

E.coli

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

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

Canada

Appendix

Appendix Notes
2.3
A549 cells are a human pulmonary type II epithelial cell line derived from a human adencarcinoma. We obtained it from ATCC about 3 years ago.

H441 is a human Clara cell line (brochiolar) We obtained it from Dr. Sheldon Feinstein whol believe obtained it from ATCC.

T7 cells were obtained from Dr Martin Post who obtained them from Dr Daphe de Mello who established this cell line from transgenic mice bearing a temperature sensitive SV viral large T antigen. It was designated a Type II cell line which produces pulmonary surfactant.

In Vitro Cell. Dev. Biol.—Animal 36:374–382, June 2000
q 2000 Society for In Vitro Biology
1071-2690/00 $05.00+0.00

**GENERATION OF AN IMMORTAL DIFFERENTIATED LUNG TYPE-II EPITHELIAL CELL LINE FROM THE ADULT H-2K$^b$tsA58 TRANSGENIC MOUSE**

DAPHNE E. deMELLO,[1] SOHIR MAHMOUD, PHILIP J. PADFIELD, AND JOSEPH W. HOFFMANN
*Department of Pathology (D. E. dM., S. M., P. J. P., J. W. H.) and Pediatric Research Institute (D. E. dM., S. M.), Cardinal Glennon Children's Hospital, St. Louis University Health Sciences Center, St. Louis, Missouri 63104*
(Received 20 September 1999; accepted 6 December 1999)

SUMMARY
This paper describes a new fully differentiated Type-II alveolar epithelial cell line designated T$_7$, derived from transgenic H-2K$^b$-tsA58 mice, capable of being passaged as an immortalized cloned cell line in culture. H-2K$^b$-tsA58 mice harbor a temperature-sensitive (ts) mutant of the simian virus 40 (SV40) large tumor antigen (T antigen) under the control of the g-interferon (INF)–inducible mouse major histocompatibility complex H-2K$^b$ promoter. When cultured under permissive conditions (338 C and in the presence of g-INF) cells isolated from H-2K$^b$-tsA58 mice express the large T antigen, which drives the cells to proliferate. However, upon withdrawal of the g-INF and transfer of the cells to a higher temperature (398 C), T antigen expression is turned off, the cells stop proliferating and differentiate. The T$_7$ cell line is a clonal cell line originally derived from a Type-II cell-rich fraction isolated from lungs of H-2K$^b$-tsA58 mice. The T$_7$ cells form confluent monolayers, and have a polarized epithelial cell morphology with tight junctions and apical microvilli. In addition, the T$_7$ cells have distinct cytoplasmic lamellar bodies, which become more numerous and pronounced when the cells are grown under nonpermissive conditions. The T$_7$ cells synthesize and secrete phosphatidylcholine and the three surfactant proteins, SP-A, SP-B, and SP-C. The T$_7$ cell line is unique in that it is the first non–tumor-derived Type-II cell line capable of synthesizing and secreting the major components of surfactant. Based on the criteria studied, the T$_7$ cell line is phenotypically very similar to normal Type-II cells. The T$_7$ cell line, therefore, should prove a valuable experimental system to advance the study of the cell biology/physiology of surfactant metabolism and secretion as well as serve as a model for other studies of Type-II cell physiology.
*Key words:* pulmonary; surfactant; lipid; protein; secretion; synthesis.

| | |
|---|---|
| **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) |
| **Morphology:** | epithelial |


| | |
|---|---|
| **Source:** | **Organ:** embryonic kidney<br>**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]<br>transfection host (Nucleofection technology from Lonza<br>Roche FuGENE® Transfection Reagents)<br>viruscide testing [92579] |
| **Receptors:** | vitronectin, expressed |
| **Tumorigenic:** | YES |
| **DNA Profile (STR):** | Amelogenin: X<br>CSF1PO: 11,12<br>D13S317: 12,14<br>D16S539: 9,13<br>D5S818: 8,9<br>D7S820: 11,12<br>THO1: 7,9.3<br>TPOX: 11<br>vWA: 16,19 |
| **Cytogenetic Analysis:** | This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells. |
| **Age:** | fetus |
| **Comments:** | Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]<br>The line is excellent for titrating human adenoviruses.<br>The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]<br>The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768] |
| **Propagation:** | **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.<br>**Atmosphere:** air, 95%; carbon dioxide ($CO_2$), 5%<br>**Temperature:** 37.0°C<br>The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C |

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Info on Cell Line(s)

| | |
|---|---|
| **Designations:** | MLE 12 |
| **Depositors:** | JA Whitsett |
| Biosafety Level: | 2 [CELLS CONTAIN PAPOVAVIRUS ] |
| **Shipped:** | frozen |
| **Medium & Serum:** | See Propagation |
| **Growth Properties:** | adherent |
| **Organism:** | Mus musculus, transgenic (mouse, transgenic) |
| **Morphology:** | epithelial |
| **Source:** | **Organ:** lung<br>**Strain:** FVB/N<br>**Cell Type:** epithelialSV40 transformed |
| **Cellular Products:** | lung surfactant proteins B and C (SP-B, SP-C) |
| **Permits/Forms:** | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| **Isolation:** | **Isolation date:** 1992 |
| **Tumorigenic:** | Yes |
| **Age:** | 5 month old |
| **Gender:** | female |
| **Comments:** | This line was established in 1992 by Kathryn A. Wikenheiser from pulmonary tumors in a mouse transgenic for the SV40 large T antigen under the control of the promoter region of the human surfactant protein C gene.<br>The cells express the mRNA for large T antigen.<br>Lung surfactant proteins B and C were detected.<br>The cells secrete phospholipids in response to phorbol esters and ATP but not in response to forskolin. |
| **Propagation:** | **ATCC complete growth medium:** HITES medium supplemented with 2% fetal bovine serum<br><br>HITES medium with 2% fetal bovine serum is formulated at the ATCC as follows:<br>• Dulbecco's medium : Ham's F12, 50:50 mix (ATCC 30-2006)<br>• Insulin 0.005 mg/ml<br>• Transferrin 0.01 mg/ml<br>• Sodium selenite 30 nM<br>• Hydrocortisone 10 nM<br>• beta-estradiol 10 nM<br>• HEPES 10 mM<br>• L-glutamine 2 mM (in addition to that in the base medium)<br>• fetal bovine serum 2%<br>**Atmosphere:** air, 95%; carbon dioxide ($CO_2$), 5% |
| **Subculturing:** | **Protocol:**<br><br>1. Remove and discard culture medium.<br>2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.<br>3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).<br>Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.<br>4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.<br>5. Add appropriate aliquots of the cell suspension to new culture vessels.<br>6. Incubate cultures at 37°C.<br><br>**Subcultivation Ratio:** A subcultivation ratio of 1:5 to 1:10 is recommended<br>**Medium Renewal:** Twice per week |

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| | |
|---|---|
| **Designations:** | A549 |
| **Depositors:** | M Lieber |
| Biosafety Level | 1 |
| **Shipped:** | frozen |
| **Medium & Serum:** | See Propagation |
| **Growth Properties:** | adherent |
| **Organism:** | *Homo sapiens* (human) |
| **Morphology:** | epithelial |
| | PHOTO |
| **Source:** | **Organ:** lung<br>**Disease:** carcinoma |
| **Cellular Products:** | keratin |
| **Permits/Forms:** | In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. |
| **Isolation:** | **Isolation date:** 1972 |
| **Applications:** | transfection host (Nucleofection technology from Lonza<br>Roche FuGENE® Transfection Reagents) |
| **DNA Profile (STR):** | Amelogenin: X,Y<br>CSF1PO: 10,12<br>D13S317: 11<br>D16S539: 11,12<br>D5S818: 11<br>D7S820: 8,11<br>THO1: 8,9.3<br>TPOX: 8,11<br>vWA: 14 |
| **Cytogenetic Analysis:** | This is a hypotriploid human cell line with the modal chromosome number of 66, occurring in 24% of cells. Cells with 64 (22%), 65, and 67 chromosome counts also occurred at relatively high frequencies; the rate with higher ploidies was low at 0.4%. There were 6 markers present in single copies in all cells. They include der(6)t(1;6)(q11;q27); ?del(6)(p23); del(11)(q21); del(2)(q11); M4 and M5. Most cells had two X and two Y chromosomes. However, one or both Y chromosomes were lost in 40% of 50 cells analyzed. Chromosomes N2 and N6 had single copies per cell, and N12 and N17 usually had 4 copies. |
| **Isoenzymes:** | G6PD: B |
| **Age:** | 58 years |
| **Gender:** | male |
| **Ethnicity:** | Caucasian |
| **Comments:** | This line was initiated in 1972 by D.J. Giard, et al. through explant culture of lung carcinomatous tissue from a 58-year-old Caucasian male. [23218]<br>Further studies by M. Lieber, et al. revealed that A549 cells could synthesize lecithin with a high percentage of desaturated fatty acids utilizing the cytidine diphosphocholine pathway. [58030]<br>The cells are positive for keratin by immunoperoxidase staining. |
| Propagation: | **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.<br>**Atmosphere:** air, 95%; carbon dioxide (CO2), 5%<br>**Temperature:** 37.0°C |
| **Subculturing:** | **Protocol:**<br>1. Remove and discard culture medium.<br>2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. |

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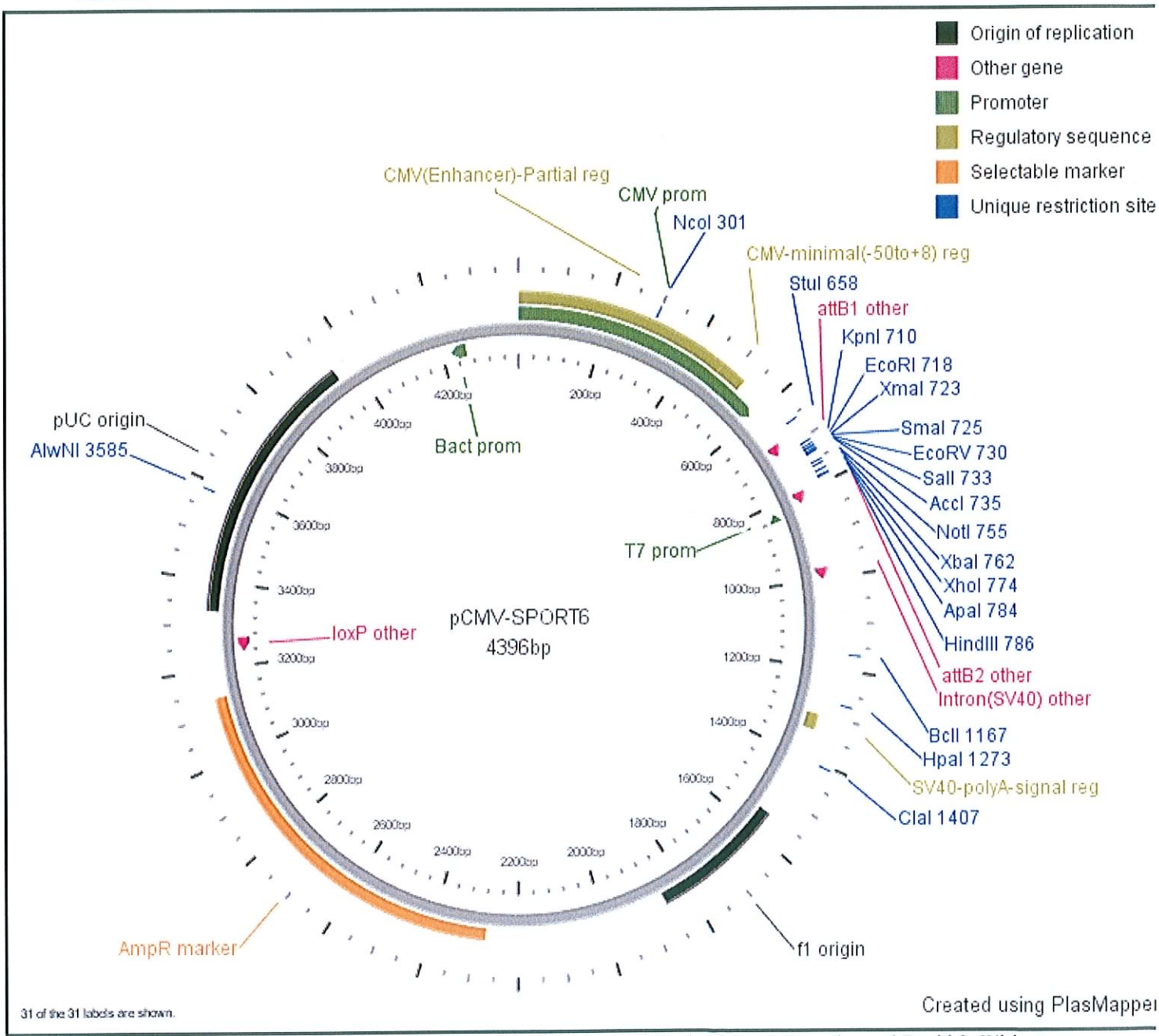
Product Information Sheet

## 4.3A pCMV-SPORT6

Vector pCMV-SPORT6

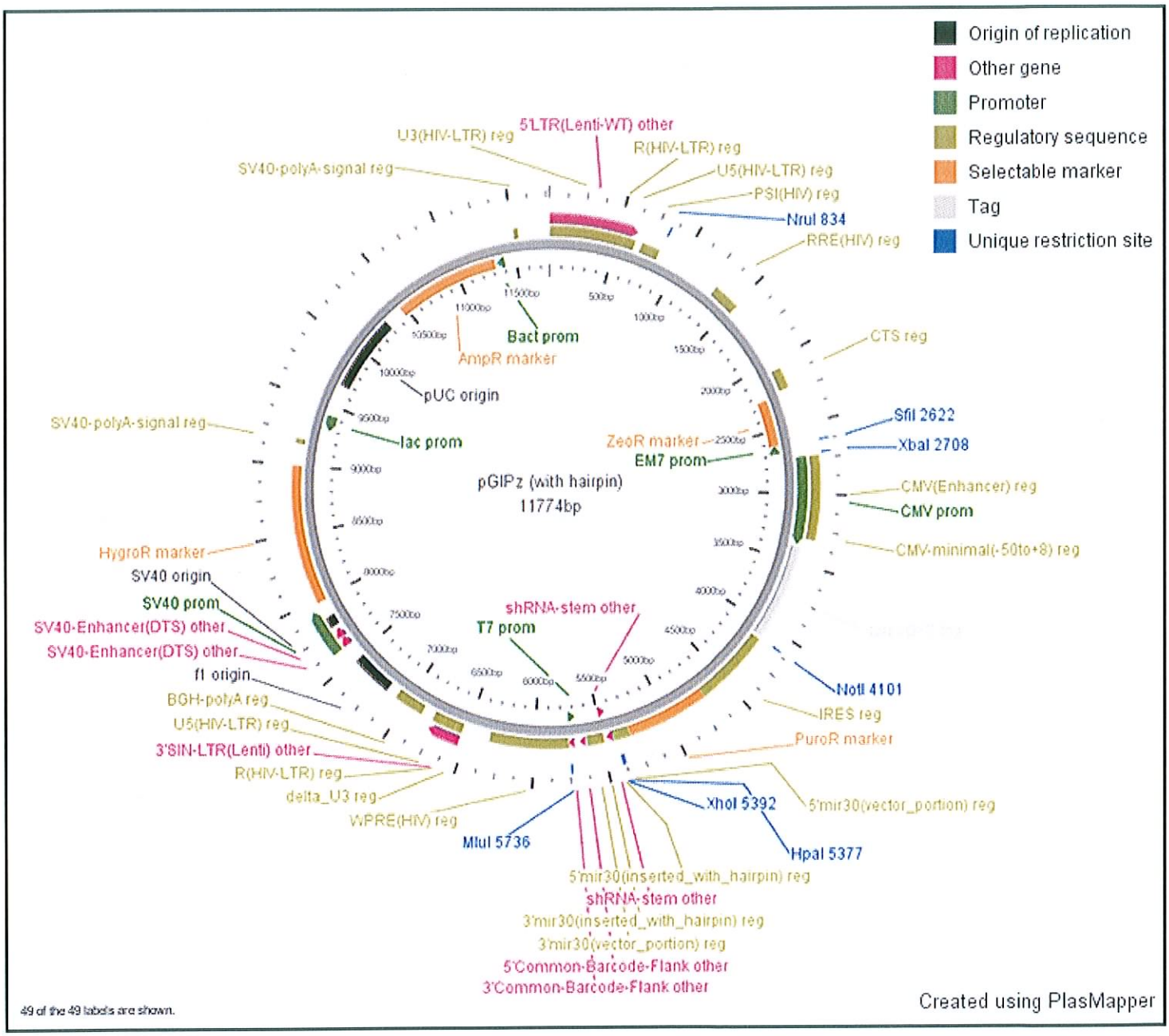
- Properties
- Vector Map
- Sequence
- Genbank
- Text Map
- Features




Vector diagram produced with PlasMapper by Xiaoli Dong, Paul Stothard, Ian J. Forsythe, and David S. Wishart
'PlasMapper: a web server for drawing and auto-annotating plasmid maps' Nucleic Acids Res. 2004 Jul 1;32(Web Server issue):W660-4.

Printer Friendly View

## 4.3B pGIPZ



## 4.3C pLKO.1

### Vector Information pLK0.1

The pLKO.1 HIV-based lentiviral vector (Figures 1-2, Table 1) allows for transient and stable transfection of shRNA and also the production of viral particles using lentiviral packaging cell lines. Stable cell lines can be selected using the puromycin selectable marker.

Human U6 Promoter RNA generated with four uridine overhangs at each 3' end

hPGK Human phosphoglycerate kinase promoter

PuroR Puromycin mammalian selectable marker

3' SIN LTR 3' self inactivating long terminal repeat (Shimada, *et al.* 1995)

f1 ori f1 origin of replication

AmpR Ampicillin bacterial selectable marker

5'LTR 5' long terminal repeat

RRE Rev response element

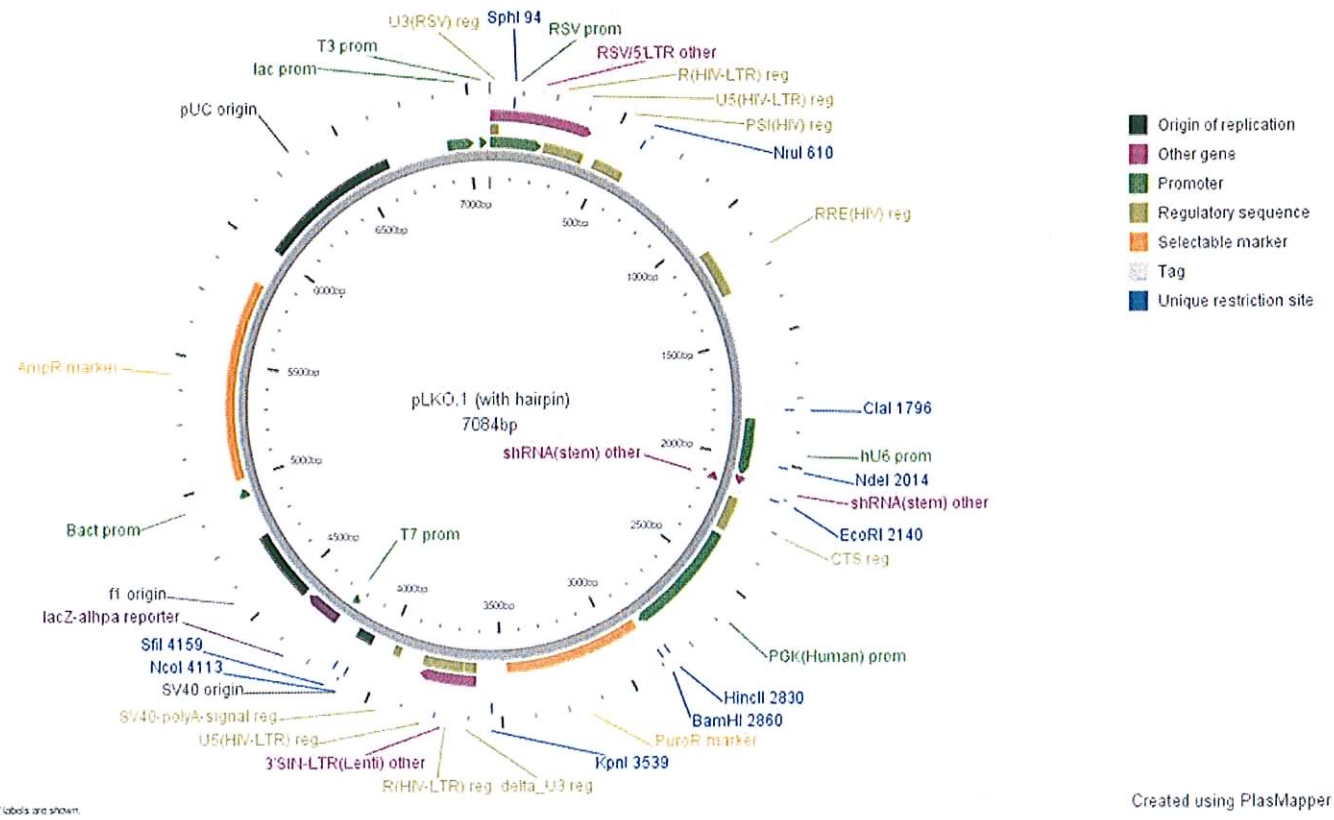
cPPT Central polypurine tract

**Table 1.** Features pLKO.1 vector

### Vector Map

**Figure 1**. The pLKO.1 vector

**Figure 2.** Map of the pLKO.1 vector




2

8.2A

Cholera Toxin from *Vibrio cholerae* ~95% (SDS-PAGE), lyophilized powder, $1 \times 10^5$-$1 \times 10^6$ units/mg protein Toxin consisting of an A subunit (27 kDa) surrounded by five B subunits (approximately 12 kDa each), which attach the toxin to ganglioside $GM_1$ on the cell surface. The A subunit catalyzes ADP-ribosylation of the α-subunit of the stimulatory G protein ($G\alpha_s$), reducing GTPase activity and activating the α-subunit. This activation of $G\alpha_s$ leads to an increase in the activity of adenylyl cyclase, resulting in increased levels of cAMP. Also ADP-ribosylates transducin in the eye rod outer segments, inactivating its GTPase activity. Cholera toxin has also been reported to ADP-ribosylate tubulin. Shown to be a potent mucosal vaccine adjuvant, inducing T helper cell type 2 responses by inhibiting the production of interleukin-12.

8.2B

Pertussis toxin from *Bordetella pertussis* buffered aqueous glycerol solution Pertussis toxin catalyzes the ADP-ribosylation of the α subunits of the heterotrimeric guanine nucleotide regulatory proteins $G_i$, $G_o$, and $G_t$. This prevents the G protein heterotrimers from interacting with receptors, thus blocking their coupling and activation. Since the $G_\alpha$ subunits remain in their GDP-bound, inactive state, they are unable to inactivate adenylyl cyclase or open $K^+$ channels.

Toxin Info

Western

## TOXIN USE RISK ASSESSMENT

| | |
|---|---|
| **Name of Toxin:** | Cholera |
| **Proposed Use Dose:** | 10 µg |
| **Proposed Storage Dose:** | 500 µg |
| **$LD_{50}$ (species):** | 250 µg |

**Calculation:**

250 µg/kg x 50 kg/person

Dose per person based on $LD_{50}$ in µg = 12500

**$LD_{50}$ per person with safety factor of 10 based on $LD_{50}$ in µg =** 1250

**Comments/Recommendations:**

Western

# TOXIN USE RISK ASSESSMENT

| | |
|---|---|
| **Name of Toxin:** | Pertussis |
| **Proposed Use Dose:** | 5 µg |
| **Proposed Storage Dose:** | 50 µg |
| **LD $_{50}$ (species):** | 15 µg |

**Calculation:**

15 µg/kg x 50 kg/person

Dose per person based on LD $_{50}$ in µg = 750

**$LD_{50}$ per person with safety factor of 10 based on $LD_{50}$ in µg =** 75

**Comments/Recommendations:**