Modification Form for Permit BIO-LRCC-0013

Permit Holder: Gabriel DiMattia

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)

Yudith Valdes

Rohann Correa

Additional Personnel
(Please list additional personnel and their Biosafety training dates here)

	Please stroke out any approved Biological Agent(s) to be removed	**Write additional Biological Agent(s) for approval below. Give the full name**
Approved Microorganisms	E. coli (DH5 alpha)	
Approved Primary and Established Cells	Human (primary), ovarian cancer patient ascites and solid tumour cells, rodent (primary), mouse embryonic fibroblasts and mouse granulosa cells. Human (established), T-47D, MCF-7, MCF-10A, ZR-75-1, Vose-14,	
Approved Use of Human Source Material	Human Blood (whole) or other Body Fluid (LHSC), Human Organs or Tissues (Unpreserved) (LHSC).	
Approved Genetic Modifications (Plasmids/Vectors)	[Plasmid]: pcDNA3.0, Pbksii, pGL3-Basic. [Gene transfected]: STC1, STC2 Plasmid 8592: pBABE-FLAG-LKB1 Plasmid 8593: pBABE-FLAG-KD LKB1	Plasmid 35097: ampkar Plasmid 20595: pWZL Neo Myr Flag PRKAA1 Plasmid 31700: SnailHA_pTK_CMV
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: Dec 16, 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:** _____

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: com.apple.ubiquity.peer-uuid.77524CF4-DFD5-47BC-B141-773523C0A26D

Digitally signed by com.apple.ubiquity.peer-uuid.77524CF4-DFD5-47BC-B141-773523C0A26D
DN: cn=com.apple.ubiquity.peer-uuid.77524CF4-DFD5-47BC-B141-773523C0A26D, o=CA
Date: 2012.11.26 16:36:44 -05'00'

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

Date of Last Biohazardous Agents Registry Form: Dec 16, 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:**

Signature of safety officer - Jamie Gibbings

November 26, 12

Modification Form for Permit BIO-LRCC-0013

Permit Holder: Gabriel DiMattia

DNA expression vectors to be added:
Plasmid 8592: pBABE-FLAG-LKB1
Plasmid 8593: pBABE-FLAG-KD LKB1
Plasmid 35097: ampkar
Plasmid 20595: pWZL Neo Myr Flag PRKAA1
Plasmid 31700: SnailHA_pTK_CMV

The above plasmids will be purchased from ADDGENE (http://www.addgene.org/) and will be used to transfect ovarian cancer cell lines to generate stable cell lines expressing the transgene or cDNAs indicated on the plasmid description sheets. **pBABE-FLAG-LKB1** will be used to express an epitope (FLAG) tagged LKBI (liver kinase B1) enzyme. **pBABE-FLAG-KD LKB1** is similar to the previously described LKB1 expression vector, but this form of the enzyme is modified to produce a non-functional enzyme as negative control for the fully functional enzyme. **Ampkar** plasmid will express AMP-activated protein kinase fused to a fluorescent protein so that it can be followed in terms of subcellular localization after transfection into cell lines. **pWZL Neo Myr Flag PRKAA1** will express a form of the AMP-activated protein kinase that forces the enzyme to be attached to cell membranes. **SnailHA_pTK_CMV** is an expression vector that will produce the transcription factor SNAIL, which regulates gene expression related to epithelial-mesenchymal transition.

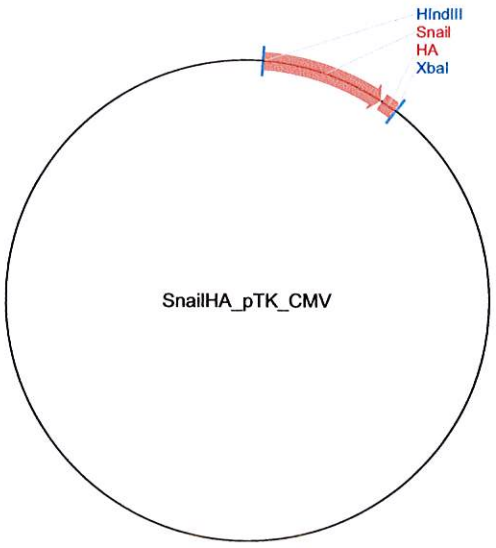
addgene
A better way to share plasmids

Plasmid 31700: SnailHA_pTK_CMV

Gene/insert name:	Snail
Alt name:	SNAI1
Insert size:	822
Species:	H. sapiens (human)
GenBank ID:	NM_005985
Entrez Gene:	SNAI1 (SLUGH2, SNA, SNAH, SNAIL, SNAIL1, dJ710H13.1)
Fusion protein or tag:	HA
Terminal:	C terminal on insert
Vector backbone:	pAdTrack-CMV (Search Vector Database)
Backbone manufacturer:	B. Vogelstein
Vector type:	Adenoviral
Backbone size w/o insert (bp):	9220
Cloning site 5':	HIndIII
Site destroyed during cloning:	Unknown
Cloning site 3':	XbaI
Site destroyed during cloning:	Unknown
5' sequencing primer:	n/a List of Sequencing Primers
Bacterial resistance(s)	Kanamycin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Sequence:	View sequences (2)
Map:	View map
Principal Investigator:	Paul Wade
Terms and Licenses:	MTA

Comments: Contains GFP

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: Aberrant expression of the transcription factors snail and slug alters the response to genotoxic stress. Kajita et al (Mol Cell Biol. 2004 Sep . 24(17):7559-66. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Ad e plasmid 31700" in your Materials and Methods section.

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A better way to share plasmids

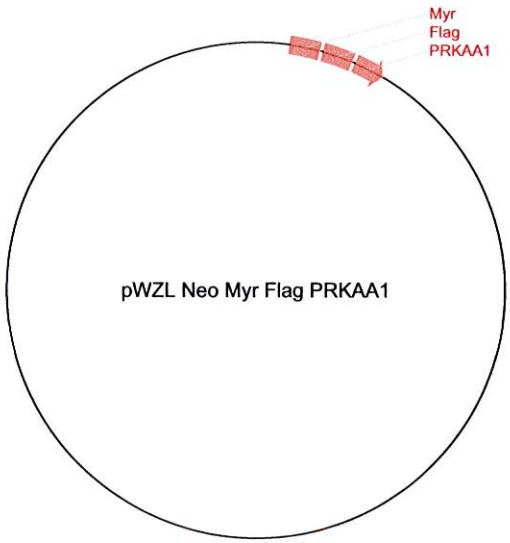
Plasmid 20595: pWZL Neo Myr Flag PRKAA1

Gene/insert name:	PRKAA1
Alt name:	protein kinase, AMP-activated, alpha 1 catalytic subunit
Species:	H. sapiens (human)
GenBank ID:	NM_0062541
Entrez Gene:	PRKAA1 (AMPK, AMPKa1)
Fusion protein or tag:	Myr
Terminal:	N terminal on backbone
Fusion protein or tag:	Flag
Terminal:	N terminal on backbone
Vector backbone:	pWZL-Neo-Myr-Flag-DEST (Search Vector Database)
Backbone manufacturer:	William Hahn Lab (available at Addgene, #15300)
Vector type:	Mammalian Expression, Retroviral
Cloning method:	Gateway Cloning
5' sequencing primer:	pWZL-Fwd (GAA CCT CCT CGT TCG ACC) List of Sequencing Primers
3' sequencing primer:	pWZL-Rev (TTC CGG GCC CTC ACA TTG)
Bacterial resistance(s)	Ampicillin
Growth strain(s)	Stbl3
Growth temperature (°C):	37
Growth instructions:	Stbl3 cells to prevent recombination.
High or low copy:	Unknown
Selectable markers:	Neomycin
Person or lab that originally cloned the gene/insert:	2-H5
Sequence:	View sequences (1)
Principal Investigator:	William Hahn
Principal Investigator:	Jean Zhao
Terms and Licenses:	MTA

Comments: ORFs were cloned into Gateway compatible pEntry vectors. An LR recombination reaction was performed to move the ORF to pWZL-Neo-Myr-Flag DEST such that the ORF would have 5' myristoylation and flag tags.

This plasmid is part of a kinase library, and the gene has been verified, but has not been fully sequenced for minor mutations. Please see the following link for plasmids from this article that are not part of the kinase library http://www.addgene.org/pubmed/17574021

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: Integrative Genomic Approaches Identify IKBKE as a Breast Cancer Oncogene (Kinase Library Clones) Boehm et al (Cell. 2007 Jun 15. 129(6):1065-1079. PubMed)

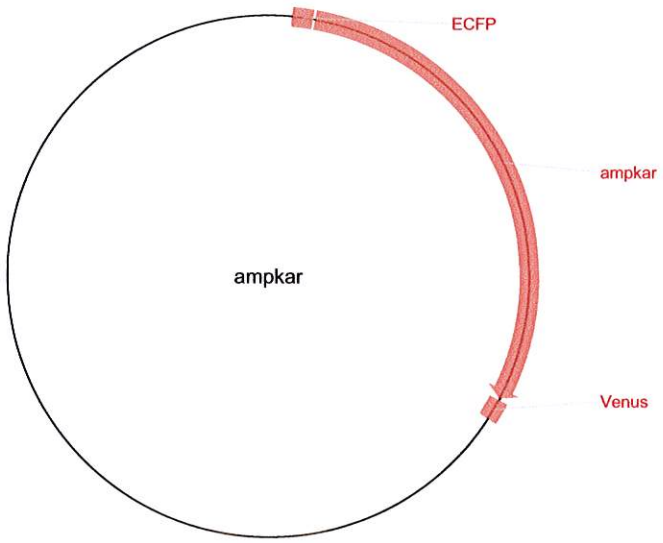
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Ad[illegible] plasmid 20595" in your Materials and Methods section.

addgene
A better way to share plasmids

Plasmid 35097: ampkar

Gene/insert name:	ampkar
Insert size:	2300
Species:	artificial
GenBank ID:	na
Fusion protein or tag:	ECFP
Terminal:	N terminal on insert
Fusion protein or tag:	Venus
Terminal:	C terminal on insert
Mutation:	na
Vector backbone:	pcDNA3 (Search Vector Database)
Vector type:	Bacterial Expression
Backbone size w/o insert (bp):	5400
Promoter:	CMV
Cloning method:	Ligation Independent Cloning
5' sequencing primer:	T7 List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	Unknown
Selectable markers:	Neomycin
Sequence:	View sequences (4)
Map:	View map
Principal Investigator:	Lewis Cantley
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: A fluorescent reporter of AMPK activity and cellular energy stress. Tsou et al (Cell Metab. 2011 Apr 6;13(4):476-86. PubMed)

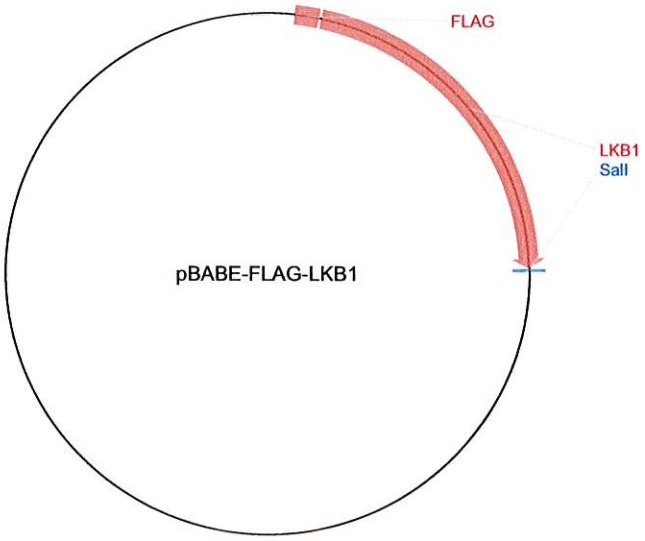
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Ad e plasmid 35097" in your Materials and Methods section.

addgene
A better way to share plasmids

Plasmid 8592: pBABE-FLAG-LKB1

Gene/insert name:	LKB1
Insert size:	1400
Species:	H. sapiens (human)
GenBank ID:	Q15831
Entrez Gene:	STK11 (PJS, LKB1)
Fusion protein or tag:	FLAG
Terminal:	N terminal on insert
Vector backbone:	pBABE-puro (Search Vector Database)
Vector type:	Mammalian Expression, Retroviral
Backbone size w/o insert (bp):	5169
Cloning site 5':	BamHI
Site destroyed during cloning:	Yes
Cloning site 3':	SalI
Site destroyed during cloning:	No
5' sequencing primer:	pBABE 5' List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Puromycin
Sequence:	View sequences (2)
Principal Investigator:	Lewis Cantley
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Shaw et al (Proc Natl Acad Sci U S A 2004 Mar 9;101(10):3329-35. Epub 2004 Feb 25. PubMed)

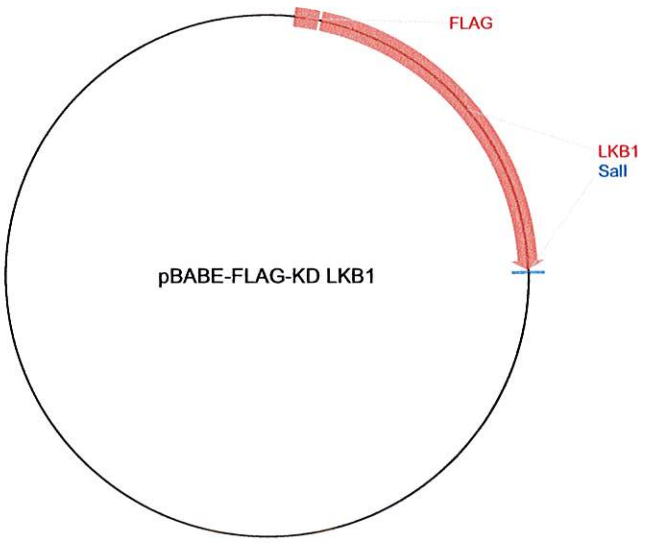
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 8592" in your Materials and Methods section.

addgene
A better way to share plasmids

Plasmid 8593: pBABE-FLAG-KD LKB1

Gene/insert name:	LKB1
Insert size:	1400
Species:	H. sapiens (human)
GenBank ID:	Q15831
Entrez Gene:	STK11 (PJS, LKB1)
Fusion protein or tag:	FLAG
Terminal:	N terminal on insert
Mutation:	K78I: lysine 78 mutated to isoleucine; kinase dead
Vector backbone:	pBABE-puro (Search Vector Database)
Vector type:	Mammalian Expression, Retroviral
Backbone size w/o insert (bp):	5169
Cloning site 5':	BamHI
Site destroyed during cloning:	Yes
Cloning site 3':	SalI
Site destroyed during cloning:	No
5' sequencing primer:	pBABE 5' List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Puromycin
Sequence:	View sequences (1)
Principal Investigator:	Lewis Cantley
Terms and Licenses:	MTA

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.



Article: The tumor suppressor LKB1 kinase directly activates AMP-activated kinase and regulates apoptosis in response to energy stress. Shaw et al (Proc Natl Acad Sci U S A 2004 Mar 9;101(10):3329-35. Epub 2004 Feb 25. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Ad e plasmid 8593" in your Materials and Methods section.

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	Gabriel DiMattia
DEPARTMENT	Oncology
ADDRESS	790 Commissioners Rd., London, ON
PHONE NUMBER	53625
EMERGENCY PHONE NUMBER(S)	519-649-4445
EMAIL	dimattia@uwo.ca

Location of experimental work to be carried out: Building(s) LRCP Room(s) A4-921; A4-908

*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: CCSRI; LRCP Small Grant

GRANT TITLE(S): CCSRI: *Implications of activated BMP signalling and Id1/Id3 function in ovarian cancer pathogenesis.* LRCP Small Grant: *PI3K/Akt/mTOR signalling & autophagy in epithelial ovarian cancer (EOC) spheroids*

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

Name	UWO E-mail Address	Date of Biosafety Training
Yudith Ramos-Valdes	yudithramos@yahoo.es	June 2011
Rohann Correa	rcorrea4@uwo.ca	October 2008

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.

Established human cell lines:
The cell lines are cancer derived lines and include human breast cancer lines T47-D, MCF-7, MCF-10A, ZR-75-1 and human ovarian cancer cell lines OVCAR3, Caov3, SkOV3, Hey, Ric2, and OW-1. When these cells are cultured they are maintained in a 37C humidified chamber with 5% CO_2 for defined periods of time after which they are harvested for extraction of protein and/or RNA. Any unused cultures are treated with bleach before disposal as biological waste using the LHSC system of waste management using biohazardous waste containers kept in the cell culture room (A4-908), which are then sealed to be autoclaved/incinerated. This includes the plastic cell culture vessels that were used to culture the cells. These lines are stored in 1.8 ml cryovials at -150C (room A4-918) when not in use.

Mouse primary cell culture:
Mouse embryonic fibroblasts generated from different mouse strains including wildtype mice and transgenic mice overexpressing STC1 or STC2 are currently stored in 1.8 ml cryovials at -150C (room A4-918). These fibroblasts have a limited lifespan in culture (~1 month) after which they are eliminated as described above for the established human cell lines. Primary cultures of mouse granulosa cells from the above strains are generated for short term experiments (e.g., 10 days) and are discarded as described above. Primary ovarian granulosa cells cannot be cryopreserved because they do not survive the freezing process.

Primary human cell cultures:
We also generate cultures of cells from the ascites fluid of ovarian cancer patients as well as primary tumour samples. These are short term cultures relative to the established cell lines; the primary human ovarian cancer cells can be kept in culture for ~1 month before they expire. These cultures are maintained in a humidified cell culture chamber as described above for defined periods of time after which they are harvested for extraction of protein and/or RNA. Any unused cultures are treated with bleach before disposal as biological waste using the LHSC system of waste management. We cryopreserve the primary human ovarian cancer cells and store aliquots of these cells in 1.8 ml cryovials at -150C (room A4-918).

Bacterial cell cultures:
The E. Coli DH5α bacterial cultures are harvested for the extraction of plasmid DNAs (pcDNA3.0 expression vector, pBKSII DNA cloning vector, pGL3-basic gene promoter analysis vector) and any unused portions of these cultures are bleached prior to disposal. The bacterial stocks are kept at -80C as 50% glycerol solutions. Agar culture plates containing the bacteria are kept at 4C sealed in parafilm and are discarded as biological waste after 2 weeks at 4C again, following the LHSC waste management system as described above. All purified plasmid DNAs are stored at -20C or -80C as aqueous solutions.

Please include a one page research summary or teaching protocol.

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 (i.e., 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 a Translational Oncology Scientist of the LRCP, 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 gynecologic 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 Translational Ovarian Cancer Research Program:

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 Research 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.

PI3K/Akt/mTOR signalling & autophagy in epithelial ovarian cancer (EOC) spheroids

This proposal is designed to test the hypothesis that the transcriptional repressor protein, TRPS1 plays an important role in mammary gland development and when present at higher than normal levels results in cellular changes that predispose the gland to neoplastic transformation. This is based on the fact that TRPS1 is overexpressed across all stages of breast cancer and that it can inhibit the activity of key regulators of mammary epithelial cell differentiated function, namely the GATA family of transcription factors and specifically, GATA3. If elevated levels of TRPS1 antagonize the role GATA3 plays in maintaining differentiated function, then it is possible that mammary epithelial cells may lose proliferative control predisposing this compartment to hyperplasia or dysplasia. To test this hypothesis we have generated transgenic mice with elevated levels of TRPS1 in the mammary gland and propose to characterize the phenotypic effects of the transgene with an emphasis on studying changes in mammary gland structure that may predict a loss of proliferative control. We will compliment these studies with human breast cancer cell

line studies where the level of TRPS1 is genetically modified and determine whether this alters the ability of these cells to grow, recover from stress, and migrate. To date there is no functional data on the role of TRPS1 in human breast cells or murine mammary gland and this proposal seeks to add significant new knowledge that will indicate whether TRPS1 is a key molecular switch governing mammary epithelial cell proliferation.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☑ 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 ☑ 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 DH5α	Yes ☑ No	Yes ☑ No	Yes ☑ No	1 litre	Stratagene	☑ 1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3
	Yes No	Yes No	Yes No			1 2 2+ 3

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☑ Yes No	Ovarian cancer patient ascites & solid tumour	Not applicable

		cells	
Rodent	☑ Yes No	Mouse embryonic fibroblasts and mouse granulosa cells	2009-023
Non-human primate	Yes ☑ No		
Other (specify)	Yes ☑ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☑ Yes ☐ No	T47-D, MCF-7, MCF-10A, ZR-75-1, vOSE-14, OVCAR3, Caov3, SkOV3, Hey, Ric2, and OW-1	Containment Level 1 for all cell lines	ATCC, Dr. Hal Hirte (McMaster University), Dr. Cheryl Conover (Mayo Clinic in Rochester, MN)
Rodent	☑ Yes ☐ No			
Non-human primate	☐ Yes ☑ No			
Other (specify)	☐ Yes ☑ No			

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) The MSDS sheets are not available for the Hey, Ric2, OW-1 (obtained from Dr. Hirte), and vOSE-14 (obtained from Dr. Conover) cell lines.

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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	LHSC	☐ Yes ☑ Unknown		O 1 ☑ 2 ☐ 2+ O 3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		O 1 O 2 ☐ 2+ O 3
Human Organs or Tissues (unpreserved)	LHSC	☐ Yes ☑ Unknown		O 1 ☑ 2 ☐ 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? ☑ 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
E. Coli DH5α	*pcDNA3.0, pBKSII, pGL3-Basic*	Promega, Invitrogen, Stratagene	*STC1, STC2*	Changes in gene expression and/or reduction in cell proliferation

* 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?
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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved? **NO**

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

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used__*Mus musculus* (mouse)_____

6.3 AUS protocol # __2009-023_____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ ☑ 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 Will live animals be used? YES No

7.3 If yes, 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

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

_____

8.0 Biological Toxins

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

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

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

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

8.5 How much of the toxin is stored*? _____

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: _____
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? 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 O "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_____ ☑ 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. ☐ 1 ☑ 2 ☐ 2+ ☐ 3

13.2 Has the facility been certified by OHS for this level of containment?
☑ YES, date of most recent biosafety inspection: Dec. 10, 2010
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

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.
Not applicable

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:

OH&S at LHSC (52286) would be contacted immediately, the injured area would be flushed with water and a disinfectant, then the injured person would be escorted to OH&SS (E1-505A) for immediate treatment (if necessary, otherwise monitored) and the appropriate accident forms completed and submitted to OH&SS at LHSC.

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: 21 June 2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: 16 Dec 2011

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: Dec 12, 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Jane Ryder
Date: July 25, 2011

Approval Number: B10-LRCC-0013 Expiry Date (3 years from Approval): December 15, 2014

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Biological Agents Registry Form: DiMattia
Date: Tue, 06 Dec 2011 11:07:45 -0500
From: Gabriel DiMattia <dimattia@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Jennifer:

E-Mail issue: Yudith doesn't have a UWO e-mail address because she's not a UWO employee (she's an LHSC technician and uses her yahoo account for everything).

Chicks: We had intended to use chick Cams in collaboration with John Lewis' lab but since he is moving to the University of Alberta, that aspect of the proposed research is no longer feasible. I have been unable to find any indication or documents showing that chick embryos could be a source of zoonotic organisms.

With regard to the cell lines: I've changed them to level 1

I hope these revision are OK.

Thank you and I apologize for my late attention to this matter.
Gabe

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

E. COLI DH5alpha MSDS

invitrogen™

Material Safety Data Sheet

Revision Date: 27-Sep-2005

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

Product code 18258012
Product name SUPERSCRIPT PLAMSID SYS WITH GATEWAY TECH (MAX EFF DH5)

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

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

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

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
dimethylsulfoxide	67-68-5	3-7

3. HAZARDS IDENTIFICATION

Emergency Overview
Irritating to eyes. Irritating to skin.

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes May cause eye irritation with susceptible persons.
Skin May cause skin irritation in susceptible persons.
Inhalation No information available
Ingestion No information available

Specific effects

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

Target Organ Effects Eyes. Skin.

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically

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

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

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

Occupational exposure controls

Exposure limits

dimethylsulfoxide	-	-	-	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

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

General Information

Form Liquid

Important Health Safety and Environmental Information

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

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

Acute toxicity

dimethylsulfoxide	14500 mg/kg (Rat)	No data available	No data available

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	No information available
Ingestion	No information available

Specific effects

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

Target Organ Effects Eyes. Skin.

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	No information available.
Bioaccumulation	No information available.

Dispose of in accordance with local regulations

IATA

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

15. REGULATORY INFORMATION

International Inventories

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

U.S. Federal Regulations

SARA 313
Not regulated

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

U.S. State Regulations

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

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

WHMIS hazard class:
D2B Toxic materials

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

16. OTHER INFORMATION

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

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

End of Safety Data Sheet

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Japan, Hong Kong, Korea, New Zealand, Singapore and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

Cell Lines

ATCC® Number: HTB-133 ™ (Order this item)

Price: $185.00

Designations: T-47D

Depositors: I Keydar

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial (PHOTO)

Source:
Organ: mammary gland; breast
Tissue: duct
Disease: ductal carcinoma
Derived from metastatic site: pleural effusion

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

Related Cell Culture Products

Receptors:	calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin; estrogen receptor, positive calcitonin, expressed androgen receptor, positive, expressed estrogen receptor, positive, expressed progesterone receptor, positive, expressed glucocorticoid receptor, positive, expressed prolactin, expressed
Tumorigenic:	Yes, forms colonies in soft agar
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 12 D16S539: 10 D5S818: 12 D7S820: 11 THO1: 6 TPOX: 11 vWA: 14
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number is 65 occurring at 50% and polyploidy at 0.8%. 18 marker chromosomes are common to most cells, of which 7 are paired and 11 are single-copied. The t(8q14q), t(9q17q), t(10q17p) are among 7 paired markers common to most cells. N7, N9, and N10 are absent and N11 is generally present in 4 copies. DM's occurred, but infrequently. Q-band examination did not show the presence of a Y chromosome.
Isoenzymes:	AK-1, 1; ES-D, 2; G6PD, B; GLO-I, 1-2; PGM1, 1; PGM3, 1
Age:	54 years adult
Gender:	female
Comments:	The cells express the WNT7B oncogene [PubMed: 8168088]. The T-47 line was isolated by I. Keydar from a pleural effusion obtained from a 54 year old female patient with an infiltrating ductal carcinoma of the breast. This differentiated epithelial substrain (T-47D) was found to contain cytoplasmic junctions and receptors to 17 beta estradiol, other steroids and calcitonin.
Propagation:	**ATCC complete growth medium:** RPMI 1640 medium with 2 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate and supplemented with 0.2 Units/ml bovine insulin, 90%; fetal bovine serum, 10% **Temperature:** 37.0C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 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. 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). 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. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation ratio:** A subcultivation ratio of 1:3 to 1:5 is recommended **Medium renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	32 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2001 recommended serum: ATCC 30-2020 purified DNA: ATCC HTB-133D purified RNA: ATCC HTB-133R
References:	1120: Judge SM , Chatterton RT Jr. Progesterone-specific stimulation of triglyceride biosynthesis in a breast cancer cell line (T-47D). Cancer Res. 43: 4407-4412, 1983. PubMed: 6871874

Cell Biology

ATCC® Number:	HTB-22™ Order this Item	Price
Designations:	MCF7	
Depositors:	CM McGrath	
Biosafety Level:	1	
Shipped:	frozen	
Medium & Serum:	See Propagation	
Growth Properties:	adherent	
Organism:	*Homo sapiens* (human)	
Morphology:	epithelial 	
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial	
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5	
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.	
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)	
Receptors:	estrogen receptor, expressed	
Antigen Expression:	Blood Type O; Rh+	
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15	
Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.	
Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1	
Age:	69 years adult	
Gender:	female	
Ethnicity:	Caucasian	
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.	
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: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% . **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C	
Subculturing:	**Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. **Note:** if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures. 1. Remove culture medium to a centrifuge tube. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 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). 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 37C to facilitate dispersal.	

ATCC® Number:	**CRL-10317™** Order this Item **Price:**
Designations:	MCF 10A
Depositors:	Michigan Cancer Foundation
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** fibrocystic disease **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** August 22, 1984
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,12 D13S317: 8,9 D16S539: 11,12 D5S818: 10,13 D7S820: 10,11 THO1: 8,9.3 TPOX: 9,11 vWA: 15,17
Isoenzymes:	AK-1, 1 [23084] ES-D, 1 [23084] G6PD, B [23084] GLO-I, 1-2 [23084] PGM1, 1-2 [23084] PGM3, 1 [23084]
Age:	36 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF 10A cell line is a non-tumorigenic epithelial cell line. [21968] The line was produced by long term culture in serum free medium with low Ca++ concentration. [21968] MCF 10A was derived from adherent cells in the population. [21968] Cells derived from a floating population are available (see MCF 10F, ATCC CRL-10318). [21968] The cells are positive for epithelial sialomucins, cytokeratins and milk fat globule antigen. [21968] They exhibit three dimensional growth in collagen, and form domes in confluent cultures. [21968] Thus far, the cells have shown no signs of terminal differentiation or senescence. The line is responsive to insulin, glucocorticoids, cholera enterotoxin, and epidermal growth factor (EGF). [21968] By electron microscopy the cells display characteristics of luminal ductal cells but not of myoepithelial cells. [23085] They also express breast specific antigens as detected by positive reaction with MFA-Breast and MC-5 monoclonal antibodies. [23085] The calcium content of the medium exerts a strong effect on the morphology of the cells. [22248]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line (MEBM) along with the additives can be obtained from Lonza/Clonetics Corporation as a kit: MEGM, Kit Catalog No. CC-3150. ATCC does not use the GA-1000 (gentamycin-amphotericin B mix) provided with kit. To make the complete growth medium, you will need to add the following components to the kit (sold separately): • 100 ng/ml cholera toxin Note: Do not filter complete medium **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO2), 5%
Subculturing:	**Protocol:** Remove medium and rinse monolayer with PBS (ATCC Cat# 30-2200).Add 3.0 ml 0.05% trypsin, 0.53 mM EDTA and incubate at 37C for 15 minutes. To neutralize trypsin, add 3 ml solution of 0.1% soybean trypsin inhibitor.Centrifuge cell suspension at 125 xg for 5 to 10 minutes.Resuspend cell pellet in complete culture medium. Add appropriate aliquots of cell suspension to new culture vessels. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:4 is recommended **Medium Renewal:** Every 2 to 3 days

LISTED CELL LINE INFORMATION

ATCC® Number:	CRL-1500™ Order this Item
Designations:	ZR-75-1
Depositors:	LW Engel
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Tissue:** duct **Disease:** ductal carcinoma **Derived from metastatic site:** ascites **Cell Type:** epithelial
Cellular Products:	mucin (apomucin, MUC-1, MUC-2)
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.
Receptors:	estrogen receptor, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,11 D13S317: 9 D16S539: 11 D5S818: 13 D7S820: 10,11 THO1: 7,9.3 TPOX: 8 vWA: 16,18
Cytogenetic Analysis:	This human cell line has a hypertriploid chromosome number. The modal chromosome number was 72 occurring in 26% of the cells and the rate of higher ploidies was at 1.2%. Eighteen markers were common.They are: t(1q,?), M2,M3, del(3) (p21), der(5)t(5;?) (q35;?), del(6) (q21), t(11q14q), t(11;11) (p15;q11), der(14)t(2;14) (q21;q32), t(17q,?), M13, M14, M15, M16, M17, der(8)t(8;?), t(9p,?) and 19pt. Of these M13 was paired. Normal N14 was not found. N6 was single copied, X had 3 copies and N18 had 4 copies in each cell.
Isoenzymes:	G6PD, B
Age:	63 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The cells produce high levels of MUC-1 mucin mRNA, low levels of MUC-2 mRNA but do not express the MUC-3 gene. [23102]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 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. 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). 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. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:4 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week
Preservation:	**Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	80 hrs

ATCC® Number:	[illegible] Order this item
Designations:	NIH:OVCAR-3
Depositors:	R Ozols, TC Hamilton
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** ovary **Disease:** adenocarcinoma **Cell Type:** epithelial
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** 1982
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
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
Cytogenetic Analysis:	The cell line is aneuploid human female, with chromosome counts in the sub to near-triploid range. Several normal chromosomes (N11, N13, N14, N15, N16, N17, and N22) are clearly under-represented. Many of these missing chromosomes are represented in the large number of cytogenetically altered chromosomes identified as marker chromosomes. In addition to the marker chromosomes, there are a large number of other structurally abnormal and unassignable chromosomes that are not recognized as markers. Random loss and gain of chromosomes from cell to cell are noted in the exact chromosome counts and in the analysis of the karyotypes.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1 PGM1, 1 PGM3, 1
Age:	60 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The NIH:OVCAR-3 line was established in 1982 by T.C. Hamilton, et al. from the malignant ascites of a patient with progressive adenocarcinoma of the ovary. Forms colonies in soft agar and has an abnormal karyotype. Resistant to clinically relevant concentrations of adriamycin, melphalan and cisplatin. Both cultured cells and xenografts exhibit androgen and estrogen receptors. Xenograft models have been used to show that treatment with 17 beta estradiol can induce progesterone receptors in this human ovarian carcinoma. NIH:OVCAR-3 is an appropriate model system in which to study drug resistance in ovarian cancer, and the presence of hormone receptors should be useful for the evaluation of hormonal therapy.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 20%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5%

Price:

ATCC® Number:	HTB-75 Order this item
Designations:	Caov-3
Depositors:	J Fogh
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
	
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
DNA Profile (STR):	Amelogenin: X CSF1PO: 10,13 D13S317: 12 D16S539: 9 D5S818: 12 D7S820: 10 THO1: 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
Age:	54 years
Gender:	female
Ethnicity:	Caucasian
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 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). 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. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week

Cell Biology

ATCC® Number:	HTB-77™ Order this Item **Price:**
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 THO1: 9,9.3 TPOX: 8,11 vWA: 17,18
Cytogenetic Analysis:	This is a hypodiploid human cell line. The modal chromosome number was 43, occurring in 63.3% of cells. The range was 42 to 45. The rate of higher ploidies was 32%. The del(1)(q21), der(13)t(1;?;13)(q11;?;q34), der(11)t(11;?) (q12), del(10)(q22) and 3 other marker chromosomes were common to most cells, and 3 others were found only in some cells. One N11 had the HSR segment from p11 to the distal end. The normal N10, N12, N15, N17 and N19 were absent. Others were either single or paired. There were from 1 to 6 rearranged and unassignable chromosomes. The X chromosome was either single or paired.
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 1-2 Me-2, 1 PGM1, 1-2 PGM3, 1
Age:	64 years
Gender:	female
Ethnicity:	Caucasian
Comments:	SK-OV-3 cells are resistant to tumor necrosis factor and to several cytotoxic drugs including diphtheria toxin, cis-platinum and adriamycin.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C

labLife Community

Vector Database > pGL3-Basic

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

pGL3-Basic

Promega

Non-viral

4818

RVprimer3

CTAGCAAAATAGGCTGTCCC

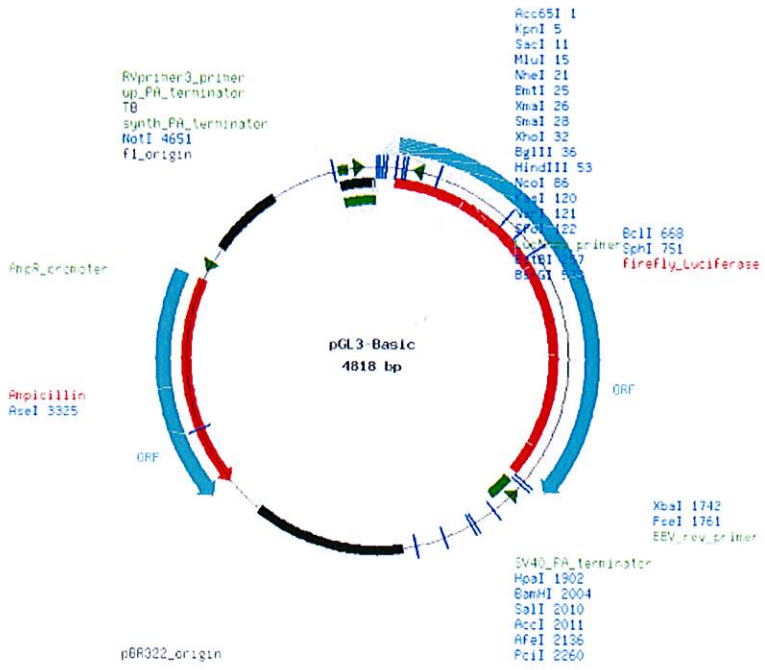
Ampicillin

Luciferase reporter vector. See http://www.promega.com/vectors/cloning_vectors.htm#b05

E1751

http://seq.yeastgenome.org/vectordb/vector_descrip/PGL3BASIC.html

View Sequence



labLife Community

Vector Database > pBlueScript SK-

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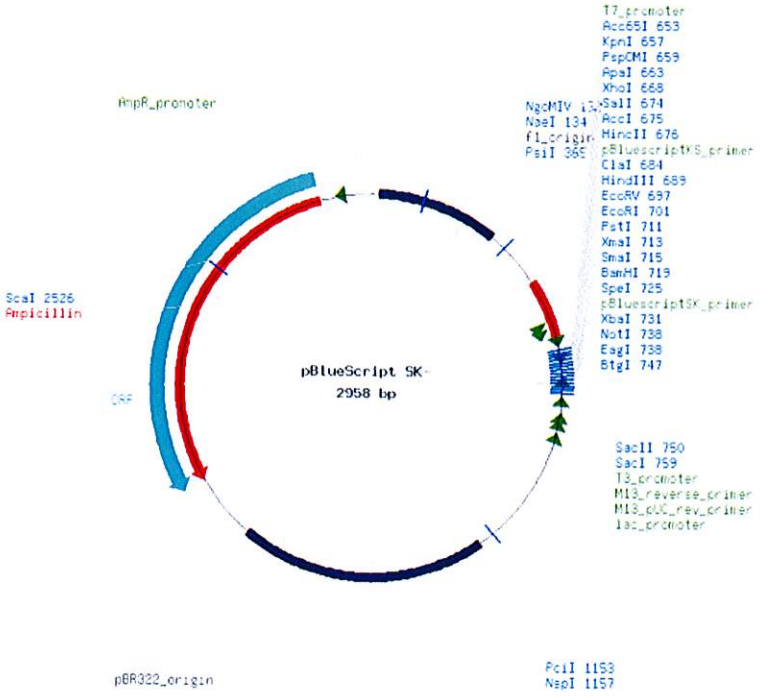
pBlueScript SK-
pBSK- , pBSSK
Stratagene
Bacterial
3000
M13/T7/T3
Amp
Discontinued
View Sequence



lablife

Vector Database > pcDNA3

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pcDNA3
Invitrogen
Mammalian expression
CMV
5446
T7
Ampicillin
Neomycin
View Sequence