Modification Form for Permit BIO-UWO-0018

Permit Holder: Peeyush Lala

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)

Elena Tutunea-Fatan

Mousumi Majumder

Lida Radan

Xiping Xin

Rabindra Bhattacharjee

Girish Gannareddy

Neena Lala

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biological Agent(s) to be removed	Write additional Biological Agent(s) for approval below. Give the full name
Approved Microorganisms	Human placental tissue samples, human placental trophoblast cell lines HTR-Svneo RSVT-2C, Choriocarcinoma cell lines Jeg3, JaR, MDA-MB-231, MCF-7, Hs57T, SKBR-3, T47D, MDA-MB-468 human breast cancer	plasmid 1771, Addgene.org pBABE-puro-hTERT www.addgene.org/1771/
Approved Primary and Established Cells	human [established]: HTR-8/Svneo, RSVT-2C human placental trophoblast cell lines, JEG3 and JaR choriocarcinoma cell line, MDA-MB-231, MCF-7, Hs57T, SKBR-3, T47D, MDA-MB-468 breast cancer cell lines,	MCF-7-COX-2 and SKBR3-COX-2 human breast cancer cell lines
Approved Use of Human Source Material	Human organs or tissues (unpreserved): Human placenta	
Approved Genetic Modifications (Plasmids/Vectors)	[bacteria]: E. coli. [plasmids]: Human Cox-2 cloned into the eukaryotic expression vector pIRES2-EGFP, SV 40 large T antigen	
Approved Use of Animals	Mice	
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:** 2

Date of Last Biohazardous Agents Registry Form: Sep 17, 2010

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:**

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

Material: Plasmid 1771, Addgene.org; pBASE-puro-hTERT

www.addgene.org/1771

Plasmid storage: -20^{0}C

Plasmid transformation in DH5α E.Coli bacteria and has marker for Puromycin, the transformed plasmid

Containing E.Coli bacteria will be grown in the Puromycin containing LB media. The E.Coli is cultured and treated with the plasmid extraction chemicals to extract the plasmid, the E.Coli get destroyed and

all the lab glass ware/plastic ware working with the E.Coli will be dipped in bleach for 15 min and later washed or discarded.

The Transformed DH5α will be stored at -80^{0}C in Glycerol stock.

Disposal: By treating with the bleach.

Materials: MCF-7-COX-2 and SKBR-3-COX-2 human breast cancer cell lines were derived by stable transfect ion of MCF-7 and SKBR-3 cell lines. They have been tested as mycoplsma free. These lines are maintained in culture and stocks are stored in liquid nitrogen. Immuno-deficient nude mice will be injected SC to test tumor development.

addgene
A better way to share plasmids

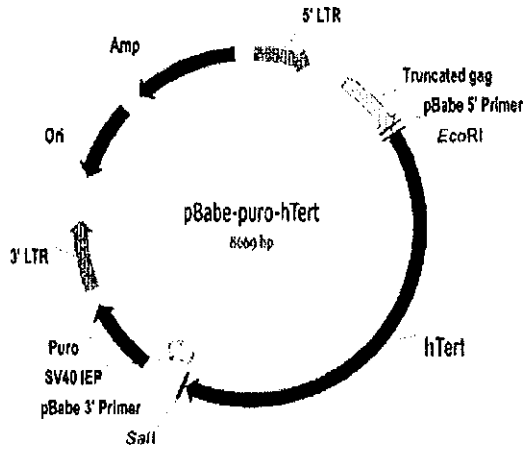
Browse > Bob Weinberg > Counter et al > pBABE-puro-hTERT

Plasmid 1771: pBABE-puro-hTERT

Gene/insert name:	hTERT
Alt name:	telomerase reverse transcriptase
Insert size:	3500
Species:	H. sapiens (human)
Entrez Gene:	TERT (DKCA2, DKCB4, EST2, TCS1, TP2, TRT, hEST2, hTRT)
Vector backbone:	pBABE-puro (Search Vector Database)
Vector type:	Mammalian Expression, Retroviral
Backbone size w/o insert (bp):	5169
Cloning site 5':	EcoRI
Site destroyed during cloning:	No
Cloning site 3':	SalI
Site destroyed during cloning:	No
5' sequencing primer:	pBABE 5' List of Sequencing Primers
3' sequencing primer:	pBABE-3
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Puromycin
Sequence:	View sequences (3)
Map:	View map
Map:	View map
Map:	View map
Supplemental document:	pBABE protocol (application/pdf)
Supplemental document:	Notes from Addgene (1)
Principal Investigator:	Bob Weinberg
Terms and Licenses:	MTA

Comments: Please note that there is a point mutation at base 1547, from A to G. This changes a glycine to an aspartate. Thus far it appears to behave like wild-type, but extensive biochemistry has not been performed.

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



Article: Dissociation among in vitro telomerase activity, telomere maintenance, and cellular immortalization. Counter et al (Proc Natl Acad Sci U S A 1998 Dec 8;95(25):14723-8. 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 1771" in your Materials and Methods section.

Western University
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: April 13, 2012
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?	If plasmids are being used to transfect cells what is the consequence on the eukaryotic cells?
N/A	YES	ADDGENE	hTERT	N/A	N/A	N/A	primary cells immortalised

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

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

Virus/Plasmid Used for Vector Construction	Vector(s) *	Source of Vector/Plasmid	Gene(s) Transduced/ Transfected	Describe the change that results from transduction/transfection
pBABE-puro-hTERT	pBABE-puro	Addgene.org	hTERT, SV40	Primary cell immortalisation

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☒ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☐ NO ☒ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

8.0 Animal Experiments

8.1 Will live animals be used? ☐ YES ☒ NO If **NO**, please proceed to section 9.0

8.2 List animal species to be used:

8.3 AUS protocol number(s):

8.4 List the location(s) for the animal experimentation and housing:

8.5 Will any of the agents listed in Sections 1-7 be used in live animals
☒ NO ☐ YES, specify:

8.6 Will the agent(s) be shed by the animal:
☐ YES ☐ NO, please justify: N/A

8.7 Indicate the PHAC or CFIA containment level used: ☐ 1 ☒ 2 ☐ 2+ ☐ 3

9.0 Use of Animal species with Zoonotic Hazards

9.1 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 10.0

9.2 Will live animals be used? ☐ YES ☒ NO

9.3 If **YES**, please specify the animal(s) used:

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

9.4 If no live animals are used, please specify the source of the specimens:
Human first timester placental tissue

------- Original Message --------
Subject: Re: Modification Form: Lala
Date: Mon, 09 Jul 2012 21:00:07 -0400
From: Peeyush Lala <pklala@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer
We are just transfecting the pBABE-hTERT
Peeyush

On 07/09/12, **Jennifer Stanley** <jstanle2@uwo.ca> wrote:
Hi there Dr. Lala

There is a question that came out of the review -

How is the lab going to immortalize the cells?
Are you making virus or just transfecting the pBABE-hTERT?

4.3.2 is incorrect

hTERT should be listed in Section 5 as a known oncogene

Regards
Jennifer

On 7/2/2012 10:13 PM, Jennifer Stanley wrote:
>Hi Dr. Lala
>Thanks for the updated info.
>The committee member(s) are reviewing it.
>Regards
>Jennifer
--
Dr.Peeyush K Lala, MD, PhD
Professor & Past Chair, Dept of Anatomy & Cell Biology,
Professor, Dept of Oncology,
Schulich School of Medicine & Dentistry,
University of Western Ontario,
London, ON, N6A 5C1
Ph: 519-661-3015 , Fax: 519-661-3936
http://www.uwo.ca/anatomy/department/lalap/plala.html

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

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

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

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

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

PRINCIPAL INVESTIGATOR	Peeyush K Lala
DEPARTMENT	Anatomy and Cell biology
ADDRESS	Medical sciences Bldg , Rm 433
PHONE NUMBER	519-661-3015 or Ext 83015
EMERGENCY PHONE NUMBER(S)	519-472-4501 (Dr Lala), 519-850-2071 (Xiping Xin)
EMAIL	pklala@uwo.ca

Location of experimental work to be carried out: Building(s) _MSB_____ Room(s)_433_____

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

FUNDING AGENCY/AGENCIES(1) CIHR, (2) Canadian Breast Cancer Foundation (CBCF)_(3) Upjohn Canada_____
GRANT TITLE(S): (1) Biology of the feto-maternal interface; (2) Regulation of lymphangiogenesis and lymphatic metastasis in a mouse breast cancer . model. (3) Natural host defence to neoplasia and its manipulations for immunotherapy_____

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

(1) The human placenta is a highly invasive "Tumor-like" structure in which a migratory and invasive cell population known as the "Extra-villous trophoblast (EVT)" cells invades the pregnant uterus and its arteries to establish an efficient exchange of oxygen and nutrients between the maternal and fetal circulations. However, unlike tumors, placental invasion of the uterus is exquisitely controlled to maintain a healthy utero-placental homeostasis. Inadequate invasiveness of EVT cells results in poor perfusion of the placenta with maternal blood, which in turn can cause intrauterine growth restriction (IUGR) of the fetus and a serious maternal disease called pre-eclampsia. Our long standing research efforts have been to identify molecular mechanisms regulating EVT cell invasiveness and their

derangements in preeclampsia and placenta cancer. For these studies we use occasional samples of human placenta, but more routinely human placenta-derived EVT cell lines produced by our lab many years ago (which we have been distributing world-wide to other researchers as well), and commercially available placental cancer (choriocarcinoma) cell lines. For example, we have discovered that two molecules , Transforming Growth factor (TGF) -β and a TGF-β binding proteoglycan decorin – both produced by the decidual tissue of the pregnant uterus negatively regulates EVT cell proliferation, migration and invasiveness in a mutually independent manner. We have found that our precancerous EVT cell lines as well as commercially available cancerous EVT (choriocarcinoma) cell lines (see list later) are resistant to the negative regulation by both molecules. We have discovered the genetic alterations responsible for TGF-β resistance in choriocarcinioma cells. Our current efforts using normal, precancerous and cancerous EVT cells (see list below) are to define the molecular mechanisms in decorin action and their alterations in trophoblast hyperinvasive disorders (precancer and cancer) as well as trophoblast hypoinvasive disorders such as pre-eclampsia.

(2) Breast cancer afflicts one in nine to ten women in Canada, and still accounts for the second highest cancer-related deaths in females, because of the difficulty in curing metastasis (spread to distant sites). This spread can occur to other organs via the new blood vessels recruited by the cancerous tissue to feed itself (a process called angiogenesis) or to lymph nodes via new lymphatic vessels draining the tumor site (a process called lymphangiogenesis). Spread to lymph nodes is often the earliest mode of spread of breast cancer. Our goals are to identify molecular mechanisms in blood-borne and lymphatic metastasis so that we can exploit this information to identify new therapeutic targets. To achieve our goals we use commercially available human breast cancer cell lines and also mouse breast cancer cell lines produced in our own laboratory from spontaneous mouse breast cancers. We conduct experiments in tissue culture and also in mice. We use immuno-deficient mice to transplant human breast cancer cell lines and syngeneic mice to transplant mouse breast cancer cell lines. We have discovered that expression of an enzyme Cyclo-oxygenase (COX)-2 promotes breast cancer progression and metastasis in mice and the human by activating prostaglandin-receptors on cancer cells. This is due to multiple mechanisms: inactivation of cancer fighting immune cells of the host, simulation of cancer cell migration and cancer-associated angiogenesis and lymphangiogenesis. Using human breast cancer tissues and numerous human breast cancer cell lines varying in COX-2 expression (see list below) we discovered that COX-2 is responsible for upregulation of vascular Endothelial growth factors stimulating angiogenesis and lymphangiogenesis. Therapeutic value of drugs blocking COX-2 enzyme such as celecoxib and drugs blocking certain prostaglandin receptors are currently being successfully tested in our mouse breast cancer models bearing transplants of mouse or human breast cancer cell lines expressing COX-2. These cell lines are listed later. For example, murine C3L5 cells (transplanted in C3H/HeJ mice) and human MDA-MB-231 cells (transplanted in nude mice) express high levels of COX-2 and are highly metastatic in vivo.

(3) We had shown that prostaglandin E2 produced by cancer cells or host macrophages in the cancer bearing host inactivates cancer-fighting immune lymphocytes of the host. This could be reversed by therapeutic use of prostaglandin inhibitors blocking cyclo-oxygenase enzymes (such as indomethacin, ibuprofen or Celicoxib) to activate the lymphocytes. We have used these drugs in combination with an immune cell activating cytokine interleukin (IL)-2 to cure different types of advanced mouse cancers and human cancers grown in immuno-deficient mice. Subsequently we applied this combination therapy protocol to advanced human kidney cancer and melanoma patients in a phase to clinical trial at the London regional Cancer centre (1988-92) with highly promising results. This combination therapy is now practiced as one of the standard protocols in these types of cancer patients at the NCI , USA. Currently we are testing similar immunotherpy protocols in mice bearing mouse or human breast cancer transplants. These transplants include syngeneic mouse breast cancer cell lines such as C3L5 developed in our lab, as well as xenotransplants of COX-2 expressing (PGE-2 producing) human breast cancer lines such as MDA-MB-231 cells (listed later).

Names of all personnel working under Principal Investigators supervision in this location: (All of them have taken biosafety training)

Xiping Xin, post-doctoral fellow	Rabindranath Bhattacharjee. Post-doctoral fellow

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

Gannareddy Girish, post-doctoral,fellow	Mousumi Bhattacharjee, post-doctoral fellow
Neena Lala, Graduate student	Elena Tutunea-Fatan, graduate student
Jose Tores-Garcia, undergraduate research assistant	Lida Raden, undergraduate research assistant

1.0 Microorganisms

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Human placental tssue samples	O Yes O X No	O Yes OX No	O Yes O X No	Not cultured	Local hospitals (as approved, see files)	O 1 O 2 X O 2+ O 3
Human placental trophoblast cell lines HTR-SVneo RSVT-2C,	O Yes O XNo	O Yes O x No	O Yes OX No	In mLs	Produced 18 years ago in my lab (see files)	O 1 O 2 X O 2+ O 3
Choriocarcinoma cell lines Jeg3, JaR	O Yes O X No	O Yes OX No	O Yes O XNo	In mLs	ATCC (see files)	O 1 O 2 X O 2+ O 3
MDA-MB-231, MCF-7, Hs57T, SKBR-3, T47D, MDA-MB-468 human breast cancer cell lines	O Yes O XNo	O Yes OX No	O Yes O XNo	In mLs	ATCC (see files)	O 1 O 2 X O 2+ O 3

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

C3-L5 and C10 mouse breast cancer cell lines					Produced in my lab 20 years ago (see files)	Level 1

*Please attach a Material Safety Data Sheet or equivalent from the supplier. These agents have already been approved earlier (see earlier files)

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O YES X 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 NoX		Not applicable
Rodent	O Yes O NoX		
Non-human primate	O Yes O NoX		
Other (specify)	O Yes O NoX		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O YesX O No	HTR-8/SVneo, RSVT-2C human placental trophoblast cell lines	Derived from human placenta in our lab some 18 years ago (see below)
		JEG3 and JaR choriocarcinoma cell line	ATCC
		MDA-MB-231, MCF-7, Hs57T, SKBR-3, T47D, MDA-MB-468 breast cancer cell lines	ATCC
		MDA-MB-468 and its lymphatic metastatic variant MDA-MB-468-LN Breast cancer cell lines, (currently being used for studies of molecular mechanisms in metastasis)	Dr Anne Chambers, LRCP
Rodent	O YesX O No	C3-L5 and C-10 mouse breast cancer cell lines	Derived from a spontaneous breast

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

			cancer in a C3H/HEJ mouse in our lab some 20 years ago (see below)
Non-human primate	O Yes O NoX		
Other (specify)	O Yes O NoX		

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

N.B. Cell lines derived in P.K.Lala's lab:

(1) HTR-8/SVneo Human Trophoblast Cell Line:

This cell line was derived at Dr P.K.Lala's laboratory by SV40 Tag immortalization of a short-lived primary human extravillous trophoblast (EVT) cell line HTR-8 isolated from a first trimester human chorionic villus explant in culture. This cell line has all the phenotypic and functional properties of the primary cell line except for being immortal. It is neither precancerous nor cancerous.

Reference: Graham, C.H., Hawley, T.S., Hawley, R.G., MacDougall, J.R., Kerbel, R.S., Khoo, N.K.S. and Lala, P.K. Establishment and characterization of first trimester human trophoblast cells with extended life span. Exp. Cell Res. 206: 204-211, 1993.

This cell line is recognized as the gold standard of normal EVT cells used in our past and current experiments outlined in section 1 above, currently also used by many other labs world-wide

(2) RSVT-2 C Human Trophoblast Cell line. RSVT-2 cell line was derived in a similar manner as above, but was selected by long life rather than selection for neomycin. It was found to be long-lived (living for 45 passages when it went through a crisis. A surviving clone was then propagated as RSVT-2C cell line

Both cell lines were found to be precancerous but not cancerous. We have been using this cell line as a model for precancerous trophoblast in experiments outlined in section 1 above.

References : (1)Khoo, N.K.S., Bechberger, J.F., Shepherd, T., Bond, S.L., McCrae, K., Hamilton, G.S. and Lala, P.K. SV40 Tag transformation of the normal invasive trophoblast results in a premalignant phenotype: Mechanisms responsible for hyperinvasiveness and resistance to antiinvasive action of TGFβ. Intl. J. Cancer. 77, 429-439, 1998

(2) Khoo, N.K.S., Zhang, Y., Bechberger, J.F., Bond, S.L., Hum, K. and Lala, P.K. SV40 Tag transformation of the normal invasive trophoblast results in a premalignant phenotype: II. Changes in gap junctional intecellular communication. Intl. J. Cancer. 77, 440-448, 1998.

(3) C3-L5 and C!0 mouse breast cancer cell lines:

C3-L5 cell line was derived by Dr P.K.Lala's laboratory from a spontaneous mammary tumor appearing in a retired female breeder mouse of C3/HeJ strain and showing lung metastasis in the original host as follows. A clone C3 produced from the primary tumor (1983) exhibited high ability for spontaneous lung metastasis from the subcutaneous (SC)transplants in the groin. With repeated passage over several years, the metastatic ability of C3 cells declined gradually, after which C3 cells were reselected in vivo by five cycles of SC to lung micro-metastasis (1987-88), and the resulting line C3L5 has since maintained its highly metastatic ability from SC transplant site to the lungs.

References: (1) Saarloos, M.N., Khoo, N.K.S., and Lala, P.K. Effects of cancer immunotherapy with indomethacin and IL-2 on murine hemopoietic stem cells. Cancer Research, 52, 6452-6462, 1992

(2) Lala, P.K. and Parhar, R.S. Eradication of spontaneous and experimental adenocarcinoma metastasis with chronic indomethacin and intermittent IL-2 therapy. Int. J. Cancer, 54, 667-684, 1993

C10 cell line was derived as a non-metastatic clone from the same spontaneous mammary tumor. Over the years with repeated passage, it has become weakly metastatic.

Reference: Jadeski, L.C., Hum, K.D., Chakraborty, C. and Lala, P.K. Nitric oxide promotes murine mammary tumor growth and metastasis by stimulating tumor cell migration, invasiveness and angiogenesis. Int. J. Cancer. 86, 30-39, 2000.

We have been using C3L5 cells as an in vitro model of metastatic breast cancer, and transplants of these cells in syngeneic C3H/HeJ mice are used in experiments as a preclinical model for COX-2 expressing breast cancer to understand molecular mechanisms in vascular and lymphatic metastasis and their abrogation with various therapies, as outlined in section 1

We have also been using C3L5 and C10 cells for differential gene arrays and have identified a good number of metastasis – promoting and suppressor genes, which are being tested in functional assays.

We are using MDA-MB-468 and MDA-MB-468 LN (supplied by Dr Ann Chambers at the LRCP) for in vitro experiments to understand molecular mechanisms in lymphatic metastasis.

We have given out HTR-8/SVneo human trophoblast and C3-L5 murine breast cancer cell lines to various research labs all over the world on the basis of MTAs approved by the biosafety office,UWO.

2.3 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 O 2 X 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 x O NO
If no, please proceed to Section 4.0

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	None	O Yes O No O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid	None	O Yes O No O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved) **Human placenta We use the unfixed tissue for extracting RNA to examine mRNA expression of certain invasion-regulating genes (such as decorin) by qPCR, and also fixed tissue for immunocytochemistry of protein expression, or in situ hybridization for mRNA localization of the same molecules (see section I for an outline of the studies)**	From UWO hospitals (as approved, see files). Before release, they are Confirmed as non-infectious by the Obstretician, based on the screening of the mothers for HIV or HBV	O Yes O No X O Unknown Unknown JR.		O 1 O 2 X O 2+ O 3
Human Organs or Tissues (preserved)	None	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? O YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E coli	Human *COX-2* cDNA cloned into the eukaryotic expression vector pIRES2-EGFP	*Kindly provided by Dr Michael Archer University of Toronto*	*We introduced Cyclo-oxygenase-(COX)2 gene into human MCF-7 and SKBR-3 breast cancer cell lines by electroporation*	*These cell lines (named as MCF-7-COX-2 and SKBR-3-COX-2 respectively) show more aggressive behavior such as migratory and invasive capacity and tumosphere formation in tissue culture.*

See e-mail

* Please attach a Material Data Sheet or equivalent if available. (No MDS available)

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below O NOX

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES , please specify ____ _____ O NO X
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify O NO X
- SV 40 Large T antigen O YES X --had been used to generate our HTR-8/SV neo human placental trophoblast cell line 18 years ago (see files, and documentation above)
  O NO
- E1A oncogene O YES O NO X
- Known oncogenes O YES, please specify O NO X
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO X

4.5 Will virus be replication defective? O YES N/A

4.6 Will virus be infectious to humans or animals? O YES N/A

4.7 Will this be expected to increase the containment level required? O YES N/A

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES O NOX
(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:_____

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

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 YESX O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used____Mice_____

6.3 AUS protocol # _2007-057, 2007-036_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NOX

6.5 Will the agent(s) be shed by the animal: O YES O NOX, please justify:
Tumors will me transplanted in mice with breast cancer cell lines. They will not shed cells. _____

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 O NoX If no, please proceed to section 8.0

7.2 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 O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES O NO X 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? O YES, Please provide details:_____ O 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? O YES O NO X 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 O NO X 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_____ O NO X
If no, please proceed to Section 12.0

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

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

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

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

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

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

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

SIGNATURE _____P.K.Lala_____

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 O 2 X O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus BIO-UWO-0018, MSB 433A inspected Sept 3, 2009
O NO, please certify
O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____P.K.Lala_____ Date:_____12 July 2010_____

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

_____
_____
_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury: Immediate local cleansing and sterilization with soap and running water, sqeezing the area to drain out blood, followed by the use of University health services.

_____
_____

15.0 Approvals

1) UWO Biohazard Subcommittee: SIGNATURE: _____
Date: Sept 17/10

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Sept 16/10

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

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

SIGNATURE:_____

Date:_____

Approval Number: BIO-UWO-0018 Expiry Date (3 years from Approval): Sept 16 2013

Special Conditions of Approval:

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

I did the tranfection of MCF 7 cells. I used DH5alpha E.Coli cell for transformation of plasmid (to increase volume of plasmid) and used that plasmid (with the microporator machine) to transfect MCF 7 cell.

Hope this will help you.

Sincerely
Mousumi

Section 4.2

Mousumi Majumder PhD
Post Doctoral Fellow
The University of Western Ontario
Department of Anatomy & Cell Biology
Medical Sciences Building, M430
London, Ontario
N6A 5C1 Canada
Tel.No: 519-661-2111 extn.80517 office
519-204-3741 resi.
Email: mmajumde@uwo.ca
majumder.mousumi@gmail.com

Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	Price:	**$256.00**

Designations:	MDA-MB-231
Depositors:	R Cailleau
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
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:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X CSF1PO: 12,13 D13S317: 13 D16S539: 12 D5S818: 12 D7S820: 8,9 THO1: 7,9.3 TPOX: 8,9 vWA: 15,18
Cytogenetic Analysis:	The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

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Cell Biology

ATCC® Number:	**HTB-22™** Order this Item	Price:	**$272.00**

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.

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- Bio-materials management; basic repository to complex partnership-level services

BioStandards

- Biological Reference Material and Consensus Standards for the life science community

Cell Biology

ATCC® Number: **HTB-133™** Order this Item Price: **$256.00**

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.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	calcitonin, expressed androgen receptor, expressed estrogen receptor, expressed progesterone receptor, expressed glucocorticoid receptor, positive, expressed prolactin, expressed calcitonin; androgen receptor, positive; progesterone receptor, positive; glucocorticoid; prolactin: estrogen receptor, positive
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 12 D16S539: 10 D5S818: 12 D7S820: 11 THO1: 6 TPOX: 11 vWA: 14
Cytogenetic Analysis:	

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Product Information Sheet

Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	Price:	**$272.00**

Designations:	MDA-MB-468
Depositors:	R Cailleau
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
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:** 1977
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	epidermal growth factor (EGF) transforming growth factor alpha (TGF alpha)
Tumorigenic:	Yes
Antigen Expression:	Blood Type AB; HLA Aw23, Aw30, B27, Bw35, Cw2, Cw4 (patient)
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 12 D16S539: 9 D5S818: 12 D7S820: 8 THO1: 7 TPOX: 8,9 vWA: 18
Cytogenetic Analysis:	modal number = 64; range = 60 to 67. The cell line is aneuploid human, presumably female (X, abnormal X) with most chromosome counts in the hypotriploid range.; Normal chromosomes X, N2, N3, N7, N8, N10, and N22 are clearly under-represented due to their involvement in the formation of the many marker (19) chromosomes present in this cell line.; A normal chromosome N1 (or two) is identified in each karyotype, but, in addition, regions of chromosome N1 are also present in five different marker chromosomes.; Variation is evident in the normal and marker chromosome copy number from karyotype to karyotype.

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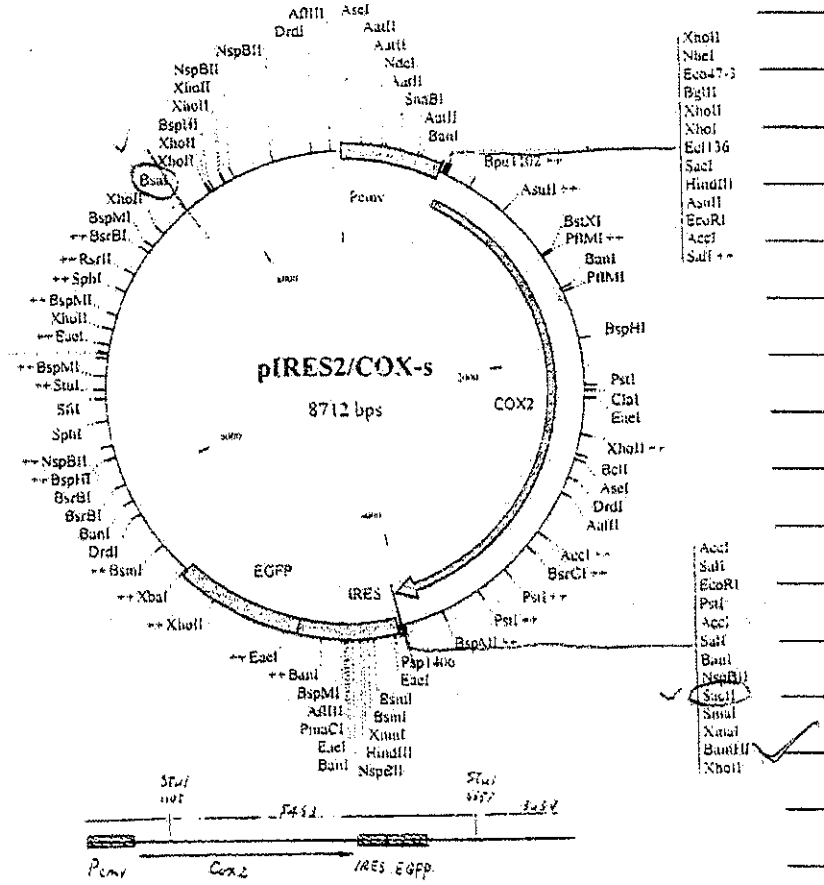
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