Modification Form for Permit BIO-UWO-0122

Permit Holder: Moshmi Bhattacharya

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)

Cameron Goertzen

Donna Cvetkovic

Cynthia Pape

~~Jeff Law~~

Magdalena Dragan

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

Julia Pasquale 04 Dec. 2012
Ryan Vanderknuk 27 Sept 12
Siddhartha Bhalla (signed up for early Jan 2013)
Gabrielle Siegers 24 Sept 2010

	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 (established): HEK 293, MDA-MB-231, MDA-MB-231shBarr1/2,MDA-MB-435S, MDA-MB-468, MCF-7, MCF-10A, MCF-12, SK-BR-3, Hs 578T, Hs 578 BST, OCVA429, JEG-3, JAR, HTR8/Svneo, MCF10aCL1	MDA-MB-231 KISS1R shRNA stable (ATCC parent 231 # HTB-26) Caco-2 (colorectal carcinoma) (=ATCC# HTB-37).
Approved Use of Human Source Material	Human Organs or Tissues [prserved]: LLSG/UH	
Approved Genetic Modifications (Plasmids/Vectors)	Plasmids: pcdna3, pEYFP, pRS, pReceiver-M13, Pegfp, pFLAG-A1	
Approved Use of Animals	Mouse	
Approved Biological Toxin(s)	Pertussis, Cholera	

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:** Parent cells = level 1 (stable line expect shRISSIR are less aggressive than parent 231's)

Date of Last Biohazardous Agents Registry Form: May 30, 2011

Date of Last Modification (if applicable): Sep 20, 2012

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:**

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?
	pRS	Origene Technologies	KISS1R				less invassive, less motile

**** 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

**** 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: mouse

8.3 AUS protocol number(s): 2012-015

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

8.5 Will any of the agents listed in Sections 1-7 be used in live animals
☐ NO ☒ YES, specify: MDA-MB-231 KISS1R shRNA

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

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:

M.Bhattacharya Permit # BIO-UWO-0122

December 2012
re: Biosafety info for shRNA KISS1R (GPR54) in breast cancer cells (MDA-MB-231)

Recent studies indicate that an increase in KISS1R and GPR54 expression in human breast tumors correlate with more advanced stages of the disease (where it conversely appears to act as a suppressor in many other human cancers)

Stable cell lines were generated using the human mammary carcinoma cell line **MDA-MB-231** (ATCC # HTB-26) parent cells electroporated with KISS1R shRNA to knock-down (k/d) but not eliminate KISS1R signaling (constructs commercially available in pRS vector; Origene Technologies). A control non-effective 'scrambled' cell line was also generated. These cell lines require the use of antibiotic (puromycin) for the cells to select for the transfected shRNA construct. (i.e. Cells with the construct will survive; once the construct is eliminated the cells will not survive in the presence of puromycine selection antibiotic). It is expected that these cells will be less aggressive/less motile/less invasive than the parent cell line, and have noted a decrease in the formation of stellate structures (3D/invasion assay) as compared to the control or parent 231 cells.

Initial work with the non-malignant MCF10a (ATCC # CRL-10317) cell line with this construct overexpressed was included in a paper published from this lab: PLos One 2011;6(6):e21599 GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness Zajac M., et.al.
A manuscript with information involving SKBR3 cells with the KISS1R construct overexpressed has been submitted to a peer reviewed journal. (Cvetcovic, D., et.al.) Nov2012

http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-26&Template=cellBiology

http://www.origene.com/shRNA/vector_information.aspx

http://www.origene.com/cdna/search-all.mspx?term=KISS1R&product=DDRNA

ATCC®

Product Information Sheet for ATCC HTB-26™

Cell Line Designation: MDA-MB-231
ATCC® Catalog No. HTB-26™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Warranty

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: mammary gland; breast adenocarcinoma; derived from metastatic site: pleural effusion
Age: 51 years
Gender: female
Ethnicity: Caucasian
Tumorigenic: yes, in nude mice also in ALS treated BALB/c mice; forms poorly differentiated adenocarcinoma (grade III)
AntigenExp: Blood Type O; Rh-
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
TH01: 7,9.3
TPOX: 8,9
vWA: 15,18

Morphology: epithelial
Growth properties: adherent
Receptors expressed: epidermal growth factor (EGF); transforming growth factor alpha (TGF alpha)
Depositors: R. Cailleau
Comments: The cells express the WNT7B oncogene [PubMed: 8168088].
Karyology: The cell line is aneuploid female, 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., Cancer Genet. Cytogenet. 3: 61, 1981.

Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA: from this line is available as ATCC Catalog HTB-26D™ (10µg)
Total RNA from this line is available as ATCC HTB-26R™ (100µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this*

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point on should be carried out under strict aseptic conditions.

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 xg for 5 to 7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a new culture flask.

5. Incubate the culture at 37°C in a suitable incubator. **(without CO2)**

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a free gas exchange with atmospheric air until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37° in a free gas exchange with atmospheric air until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM 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 with 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 cell suspension to new culture vessels.
   Subcultivation Ratio: 1:2 to 1:4.

6. Place culture vessels in incubators at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

2 to 3 times weekly.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Leibovitz's L-15 Medium, Catalog No. 30-2008.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO_2 and air mixture is detrimental to cells when using this medium for cultivation
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020.

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.

Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references are available in the catalog at www.atcc.org)

Brinkley BR et al. **Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro**. Cancer Res. 40: 3118-3129, 1980 PubMed: 81042058

Cruciger Q et al. **Morphological, biochemical and chromosomal characterization of breast tumor lines from pleural effusions**. In Vitro 12: 331, 1976

Siciliano MJ et al. **Mutually exclusive genetic signatures of human breast tumor cell lines with a**

common chromosomal marker. Cancer Res. 39: 919-922, 1979 PubMed: 79146816

Cailleau R et al. **Breast tumor cell lines from pleural effusions.** J. Natl. Cancer Inst. 53: 661-674, 1974 PubMed: 75007579

Cailleau R et al. **Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization.** In Vitro 14: 911-915, 1978 PubMed: 79087497

Bates SE et al. **Expression of the transforming growth factor-alpha/epidermal growth factor receptor pathway in normal human breast epithelial cells**. Endocrinology 126: 596-607, 1990 PubMed: 90092004

Dickstein B et al. **Increased epidermal growth factor receptor in an estrogen-responsive, adriamycin-resistant MCF-7 cell line**. J. Cell. Physiol. 157: 110-118, 1993 PubMed: 94012995

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and disease states of human breast tissue**. Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

Satya-Prakash KL et al. **Cytogenetic analysis on eight human breast tumor cell lines: high frequencies of 1q, 11q and HeLa-like marker chromosomes**. Cancer Genet. Cytogenet. 3: 61-73, 1981 PubMed: 82001960

Katayose Y et al. **Promoting apoptosis: a novel activity associated with the Cyclin-dependent kinase inhibitor p27**. Cancer Res. 57: 5441-5445, 1997 PubMed: 98069835

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma**. Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Sheng S et al. **Maspin acts at the cell membrane to inhibit invasion and motility of mammary and prostatic cancer cells**. Proc. Natl. Acad. Sci. USA 93: 11669-11674, 1996 PubMed: 97030253

De Vincenzo R et al. **Antiproliferative activity of colchicine analogues on MDR-positive and MDR-negative human cancer cell lines.** Anti-Cancer Drug Des. 13: 19-33, 1998 PubMed: 98134515

Soker S et al. **Characterization of novel vascular endothelial growth factor (VEGF) receptors on tumor cells that bind VEGF165 via its exon 7-endoded domain**. J. Biol. Chem. 271: 5761-5767, 1996 PubMed: 96215040

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**. 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods **11**:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

ATCC Warranty

The viability of ATCC products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 (U.S., Canada, and Puerto Rico) or 703-365-2700 (elsewhere) or by e-mail at tech@atcc.org.

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This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

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Product Information Sheet for ATCC® HTB-37™

Cell Line Designation: Caco-2
ATCC® Catalog No. HTB-37™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Subculturing Procedure
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: colon; colorectal adenocarcinoma
Age: 72 years
Gender: male
Ethnicity: Caucasian
Morphology: epithelial
Doubling time: about 62 hours
Growth Properties: adherent
Antigens Expressed: Blood Type O; Rh+
Virus Susceptibility: human immunodeficiency virus (HIV, LAV)
Tumorigenic: Yes, in nude mice; forms moderately well differentiated adenocarcinoma consistent with colonic primary (grade II)
Receptors Expressed: heat stable enterotoxin (Sta, E. coli); epidermal growth factor (EGF)
DNA Profile (STR analysis):

Amelogenin: X
CSF1PO: 11
D13S317: 11,13,14
D16S539: 12,13
D5S818: 12,13
D7S820: 11,12
TH01: 6
TPOX: 9,11
vWA: 16,18

Depositor: J. Fogh
Comments: This line was isolated from a primary colonic tumor. Upon reaching confluence, the cells express characteristics of enterocytic differentiation. Caco-2 cells express retinoic acid binding protein I and retinol binding protein II, and are keratin positive.
Karyology: This is a human cell line. The stemline modal chromosome number is 96, occurring at 16% with polyploidy at 3.2%. Ten common markers were detected i.e., t(1q;?), 10q-, t(11q17q) and 7 others. The t(1q17q) and M11 were found in a portion of cells. The ins(2), 10q-, and t(15q;?) were generally paired, and t(11q;17q) and t(21q;?) were mostly three-copied. Normal N9 was absent, and N21 was lost in some cells. One to 4 small acrocentric chromosomes were detected. No Y chromosome with bright distal q-band was detected by Q-observation.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These 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.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

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Or contact your local distributor.

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. Discard supernatant.
4. Resuspend the cell pellet with the recommended complete medium and dispense into a 25 cm^2 culture flask. It is important to avoid excessive alkalinity of the medium during recovery of the cells. *It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for a 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

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 10 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. Recommended inoculum 1x10(4) viable cells/cm^2.
   Subcultivation Ratio: 1:4 to 1:6.

6. Incubate cultures at 37°C.

7. Subculture cells when they are about 80% confluent, at a cell concentration between 8x10(4) and 1 x 10(5) cells/cm^2.

   Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in ***Culture of Animal Cells: A Manual of Basic Technique*** by R. Ian Freshney, 4th edition, published by Wiley - Liss, N.Y., 2000.

Medium Renewal

Fluid change cells every 4 to 5 days.

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 20%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC® Catalog No. 30-2020 (500ml) and ATCC® Catalog No. 30-2021(100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO. Cell culture tested DMSO is available as ATCC® Catalog No. 4-X.

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Or contact your local distributor.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

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Modification Form for Permit BIO-UWO-0122

Permit Holder: Moshmi Bhattacharya

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)

~~Josh Burley~~

Donna Cvetkovic

Cynthia Pape

Jeff Law

Magdalena Dragan

Additional Personnel
(Please list additional personnel here)

Cameron Goetzen

	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 (established): HEK 293, MDA-MB-231, MDA-MB-231shBarr1/2,MDA-MB-435S, MDA-MB-468, MCF-7, MCF-10A, MCF-12, SK-BR-3, Hs 578T, Hs 578 BST, OCVA429, JEG-3, JAR, HTR8/Svneo, MCF10aCL1	SKBR3 pFlagA1 SKBR3 Fl-KISS1R (ATCC # HTB-30 parent line)
Approved Use of Human Source Material	Human Organs or Tissues [prserved]: LLSG/UH	
Approved Genetic Modifications (Plasmids/Vectors)	Plasmids: pcdna3, pEYFP, pRS, pReceiver-M13, Pegfp, pFLAG-A1	
Approved Use of Animals	Mouse	
Approved Biological Toxin(s)	Pertussis, Cholera	

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: May 30, 2011

Date of Last Modification (if applicable): Apr 16, 2012

BioSafety Officer(s)*: J Stanley Sept 14/12

***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:** 20 Sept 2012

M.Bhattacharya Permit # BIO-UWO-0122

JULY 2012
re: Biosafety info for Fl-KISS1R (GPR54) in breast cancer cells (SKBR3)

Recent studies indicate that an increase in KISS1R and GPR54 expression in human breast tumors correlate with more advanced stages of the disease (where it conversely appears to act as a suppressor in many other human cancers)

Stable cell lines were generated using the human mammary adenocarcinoma cell line SKBR3 (ATCC # HTB-30) parent cells microporated with FLAG-KISS1R (GPR54) or empty vector control pFLAG A1 (vector has pEGFP-C3 backbone). These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells in media without the antibiotic will expel the construct.) When stimulated with ligand the KISS1R stable cell line shows significant increase in cell motility (2D/migration assay), and increase in the formation of stellate structures (3D/invasion assay) as compared to the pFlagA1 control or parent SKBR3 cells.

Initial work with the non-malignant MCF10a (ATCC # CRL-10317) cell line with this construct was included in a paper published from this lab: PLos One 2011;6(6):e21599 GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness Zajac M., et.al. A manuscript with information involving SKBR3 cells with the KISS1R construct is in preparation for submission to a peer reviewed journal. (Cvetcovic, D., et.al.) July2012

(Below previously submitted March 2012):
M.Bhattacharya Permit # BIO-UWO-0122

MARCH 2012
re: Biosafety info for Fl-KISS1R (GPR54) in breast cancer cells (MCF10a)

Recent studies indicate that an increase in KISS1R and GPR54 expression in human breast tumors correlate with more advanced stages of the disease (where it conversely appears to act as a suppressor in many other human cancers)

Stable cell lines were generated using the normal human mammary epithelial cell line **MCF10a** parent cells electroporated with FLAG-KISS1R (GPR54) or empty vector control pFLAG A1 (vector has pEGFP-C3 backbone). These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells in media without the antibiotic will expel the construct.) When stimulated with ligand the stable cell lines show significant increase in cell motility (2D/migration assay), increase in the formation of stellate structures (3D/invasion assay), and increased MMP-9 activity as compared to the parent MCF10a cells.

This information was included in a paper published from this lab: PLos One 2011;6(6):e21599
GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness
Zajac M., et.al.

Cell Line Designation: SK-BR-3
ATCC® Catalog No. HTB-30™

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: breast ;adenocarcinoma; derived from metastatic site:; malignant pleural effusion
Age: 43 years
Gender: female
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
AntigenExp: Blood Type A; Rh+; HLA A11, Bw22(+/-), B40, B18
Tumorigenic: yes, in nude mice; forms poorly differentiated adenocarcinoma
DNA profile (STR analysis)

Amelogenin: X
CSF1PO: 12
D13S317: 11,12
D16S539: 9
D5S818: 9,12
D7S820: 9,12
TH01: 8,9
TPOX: 8,11
vWA: 17

Depositors: G. Trempe; L.J. Old
Comments: This cell line was derived by G. Trempe and L.J. Old in 1970 from a pleural effusion. Ultrastructural features include microvilli and desmosomes, glycogen granules, large lysosomes, bundles of cytoplasmic fibrils. No virus particles
The SK-BR-3 cell line overexpresses the HER2/c-erb-2 gene product.
Karyotype: This is a hypertriploid human cell line with the modal chromosome number of 84, occurring in 34% of cells. Cells having 80 chromosomes also occurred at a high rate (28%); the higher ploidy cells occurred at 7.3%.
This cell line has a very complex chromosome composition. Thirty-five to 40% of chromosomes in a cell complement with a modal chromosome number of 84 consisted of structurally altered marker chromosomes. Several markers are longer than chromosome N1.
The origins of most of these markers, however, are not clear. Some markers may have at least three individual chromosome segments.
The markers [i.e., ?der(1)t(1;21) (p13;q21) [or ?t(1q21q)], ?del(2) (q13), and t(7pter--cen--?), present in some cells only] were the only ones in which portions of chromosome segments could be identified. Most cells had about three normal X chromosomes and five or more N7. The structurally normal N1, N14 and N17 were generally absent.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA: from this line is available as ATCC HTB-30D (10 micrograms)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use 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.
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) 639-3620; FAX (212) 753-5764

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-

ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

5. Incubate the culture at **37°C** in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).

2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.

3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.

2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin-0.53mM 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.
   Subcultivation ratio: 1:2 to 1:3

6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994

Medium Renewal

Two to three times weekly

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%

This medium is formulated for use with a 5% CO_2 in air atmosphere
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete culture medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at **www.atcc.org**)

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Trempe GL. **Human breast cancer in culture. Recent Results** Cancer Res. : 33-41, 1976 PubMed: 77103992

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Caputo, J. L., **Biosafety procedures in cell culture.** J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice.** Second edition, ASM press, Washington, DC.

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PFLAG A1 VECTOR MAP (BACKBONE PEGFP-C3) INFO FOR BIOSAFETY JULY & MARCH 2012:

*GCTAGC***GGGATGGACTACAAGGACGACGACGACAAG***GGATCCG**GAATTC**GG**GTCGAC**CTCGAG**GCGGCCGC**TCTAGA **pFLAG-M1**

*GCTAGC***GGGATGGACTACAAGGACGACGACGACAAG***GGATCCGGAATTCGGGGTCGACCTCGAGGCGGCCGC**TCTAGA **pFLAG-A1**

NheI Kozak FLAG BamHI EcoRI SalI XhoI NotI XbaI
(G/ANNATGG)

KISS1-R FWD gtgccagggcgcaatcctggagggcgg
KISS1-R REV ccgagggagccgctcggattcccacc

(clone into pFLAG A1)
KISS1-R FWD BHI GGGCCC*GGATCC*atgcacaccgtggctacgtccggaccc
KISS1-R REV NOTI GGGCCC*GGCGGCCGC*tcagagaggggcgttgtcctcccccagg

(clone into pEGFP-N1)
KISS1-R FWD BGLII GGGCCC*AGATCT***ACC**atgc*acaccgtggctacgtccggaccc
KISS1-R REV no stop HIII GGGCCC*GAAGCTT*gagaggggcgttgtcctcccccagg

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?
DH5alpha	pFlagA1 (PEGFPbackbone)	original Clonetech	KISS1R	no	no	no	expect to be more motile

** 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

** 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:

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:

Modification Form for Permit BIO-UWO-0122

Permit Holder: Moshmi Bhattacharya

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)

Josh Burley

Donna Cvetkovic

~~Mistre Alemayehu~~

Cynthia Pape

Jeff Law

Additional Personnel
(Please list additional personnel here)

Magda Dragon

	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 (established): HEK 293, MDA-MB-231, MDA-MB-231shBarr1/2,MDA-MB-435S, MDA-MB-468, MCF-7, MCF-10A, MCF-12, SK-BR-3, Hs 578T, Hs 578 BST, OCVA429, JEG-3, JAR, HTR8/Svneo, MCF10aCL1	mcf10a pFlag A1 (vector) mcf10a Fl-KISS1R (GPR54) MDA-MB-231 pFlag A1 (vector) MDA-MB-231 Fl-KISS1R MDA-MB-231 RalGDS mutant (616-768)
Approved Use of Human Source Material	Human Organs or Tissues [preserved]: LLSG/UH	MDA-MB-231 sh RalA MDA-MB-231 sh RalB MDA-MB-231 sh RalA/B MDA-MB-231 sh NE-GFP (Scrambled) BT-20 (ATCC HTB-19)
Approved Genetic Modifications (Plasmids/Vectors)	Plasmids: pcdna3, pEYFP, pRS, pReceiver-M13	pEGFP pFLAG-A1
Approved Use of Animals	Mouse	
Approved Biological Toxin(s)	Pertussis, Cholera	

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:** 1 or 2 (see attached)

Date of Last Biohazardous Agents Registry Form: May 30, 2011

Date of Last Modification (if applicable): _____

BioSafety Officer(s)*: J Stanley April 13, 2012

***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:** 16 April 2012

MARCH 2012
re: Biosafety info for Fl-KISS1R (GPR54) in normal and breast cancer cells

Recent studies indicate that an increase in KISS1R and GPR54 expression in human breast tumors correlate with more advanced stages of the disease (where it conversely appears to act as a suppressor in many other human cancers)

Stable cell lines were generated using the normal human mammary epithelial cell line MCF10a parent cells and human breast carcinoma cell line MDA-MB-231 parent cells electroporated with FLAG-KISS1R (GPR54) or empty vector control pFLAG A1 (vector has pEGFP-C3 backbone). These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells in media without the antibiotic will expel the construct.) When stimulated with ligand the stable cell lines show significant increase in cell motility (2D/migration assay), increase in the formation of stellate structures (3D/invasion assay), and increased MMP-9 activity as compared to the parent cells.

This information was included in a paper published from this lab: PLos One 2011;6(6):e21599
GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness
Zajac M., et.al.

→Level 1 req'd
(in vitro, in vivo) JS

MARCH 2012
re: Biosafety info for shRNA Ral A & Ral B in breast cancer cells

Investigation in our lab showed elevated mRNA levels of Ral-GTPases in advanced stages of human breast cancer.

Stable cell lines were generated using the human breast carcinoma cell line MDA-MB-231 parent cells electroporated with shRalA and/or shRalB (constructs commercially available in pRS vector; Origene Technologies). A control non-effective 'scrambled' cell line was also generated. These cell lines require the use of antibiotic (puromycin) for the cells to maintain the transfected shRNA construct. (i.e. Cells exposed to media without the antibiotic will expel the shRNA construct.) The stable cell lines show significant reduction in cell motility (2D/migration assay) as well as a decrease in the formation of stellate structures (3D/invasion assay) as compared to the parent MDA-MB-231 cells.

This information was included in a paper published from this lab: Mol Cancer Res 2009;7(7):1064–77
{beta}-Arrestin/Ral Signaling Regulates Lysophosphatidic Acid-Mediated Migration and Invasion of Human Breast Tumor Cells
Timothy T. Li, et.al.

→level 2 req'd
(in vitro, in vivo) QL

MARCH 2012
re: Biosafety info for RalGDS mutant in breast cancer cells

Investigation in our lab showed elevated mRNA levels of Ral-GTPases in advanced stages of human breast cancer.

Stable cell lines were generated using the human breast carcinoma cell line MDA-MB-231 parent cells electroporated with RalGDS mutant(616-768). A control empty vector cell line was also generated. These cell lines require the use of antibiotic (G418) for the cells to maintain the transfected construct. (i.e. Cells exposed to media without the antibiotic will expel the construct.) The stable cell lines show significant reduction in cell motility (2D/migration assay) as well as a decrease in the formation of stellate structures (3D/invasion assay) as compared to the parent MDA-MB-231 cells. ie mutant acting as an inhibitor of motility in vitro.

This information was included in a paper published from this lab: Mol Cancer Res 2009;7(7):1064–77
{beta}-Arrestin/Ral Signaling Regulates Lysophosphatidic Acid-Mediated Migration and Invasion of Human Breast Tumor Cells
Timothy T. Li, et.al.

Mod Biological Agents March 2012
Add new cell line:
BT-20 human breast carcinoma ATCC #HTB-19
http://www.atcc.org/ATCCAdvancedCatalogSearch/ProductDetails/tabid/452/Default.aspx?ATCCNum=HTB-19&Template=cellBiology

→ Level 2 required
(in vitro, in vivo) QL

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Cell Biology

ATCC® Number: HTB-19™ Order this Item

Price: $431.00 (for-profit list price)
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See New Benefits of ATCC Culture

Designations:	BT-20
Depositors:	EY Lasfargues
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens*
Morphology:	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** carcinoma
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:** 1958
Applications:	BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5. This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices.
Tumorigenic:	Yes
Reverse Transcript:	negative
Antigen Expression:	HLA A1, Bw16 (+/-)
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 11 D16S539: 11,14 D5S818: 12 D7S820: 10 THO1: 7,9.3 TPOX: 11 vWA: 16,17
Cytogenetic Analysis:	Normal chromosomes N3, N4, N9, N13, N14, and X may be absent. The markers der(11)t(11;?)(q25;?) (M1); der(1)t(1;3)(p22?;p13?) (M2); and der(2)t(2;?) (q37;?) (M5) were detected by W.A. Nelson-Rees, et al., Int. J. Cancer 16: 74-85, 1975.
Isoenzymes:	AK-1, 1-2 ES-D, 1 G6PD, B GLO-I, 1-2 PGM1, 1 PGM3, 1
Age:	74 years
Gender:	female
Ethnicity:	Caucasian
Comments:	The cells express the WNT3 and the WNT7B oncogenes [PubMed: 8168088]. This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices. A mycoplasma contaminant was discovered and eliminated early in 1972. Growth of BT-20 cells is inhibited by tumor necrosis factor alpha (TNF alpha). BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:**

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Cell Line Designation: BT-20
ATCC® Catalog No. HTB-19

Table of Contents:

- Cell Line Description
- Biosafety Level
- Use Restrictions
- Handling Procedure for Frozen Cells
- Handling Procedure for Flask Cultures
- Medium Renewal
- Complete Growth Medium
- Cryoprotectant Medium
- References
- Replacement Policy
- Specific Batch Information

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: carcinoma; mammary gland; breast
Age: 74 years
Gender: female
Ethnicity: Caucasian
Growth Properties: adherent
Morphology: epithelial
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 12
D13S317: 11
D16S539: 11,14
D5S818: 12
D7S820: 10
TH01: 7,9.3
TPOX: 11
vWA: 16,17
Tumorigenic: yes, in nude mice; forms grade II adenocarcinomas
Reverse Transcriptase: negative
AntigenExp: HLA A1, Bw16 (+/-)
Depositors: E.Y. Lasfargues
Passage submitted to the ATCC: 248
Comments: This breast tumor line was established by E.Y. Lasfargues and L. Ozzello in 1958 by isolation and cultivation of cells spilling out of the tumor when it was cut in thin slices. A mycoplasma contaminant was discovered and eliminated early in 1972. Growth of BT-20 cells is inhibited by tumor necrosis factor alpha (TNF alpha). BT-20 cells are negative for estrogen receptor, but do express an estrogen receptor mRNA that has deletion of exon 5. The cells express the WNT3 and the WNT7B oncogenes [PubMed: 8168088].
Cytogenetic analysis: Normal chromosomes N3, N4, N9, N13, N14, and X may be absent. The markers der(11)t(11;?)(q25;?) (M1); der(1)t(1;3)(p22?;p13?) (M2); and der(2)t(2;?) (q37;?) (M5) were detected by W.A. Nelson-Rees, et al., Int. J. Cancer 16: 74-85, 1975.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens. ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., 1995) the ATCC manual on quality control (Hay et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, ***Biosafety in Microbiological and Biomedical Laboratories*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*
3. Transfer the vial contents to a centrifuge tube containing 9.0 ml complete culture medium, and spin at approximately 125 xg for 5 to7 minutes.

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Product Information Sheet for HTB-19

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio), and dispense into a 25 cm^2 or a 75 cm^2 culture flask. *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product.

Handling Procedure for Flask Culture;

The flask was seeded with cells (see specific batch information) grown and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination. Also check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable).
2. **If the cells are still attached,** aseptically remove all but 5 to 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO_2 in air atmosphere until they are ready to be subcultured.
3. **If the cells are not attached,** aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to 25 cm^2 flask. Incubate at 37°C in a 5% CO_2 in air atmosphere until cells are ready to be subcultured.

Subculturing Procedure

Volumes used in this protocol are for 75 cm^2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

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.
Subcultivation Ratio: 1:2 to 1:6
6. Incubate cultures at 37°C.

Note: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 10 in ***Culture of Animal Cells, a manual of Basic Technique*** by R. Ian Freshney, 3rd edition, published by Alan R. Liss, N.Y., 1994.

Medium Renewal

Two to three times weekly

Complete Growth Medium

The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003.
To make the complete growth medium, add the following components to the base medium:

- fetal bovine serum to a final concentration of 10%

This medium is formulated for use with a 5% CO_2 in air atmosphere.
ATCC tested fetal bovine serum is available as ATCC Catalog No. 30-2020 (500ml) and ATCC Catalog No. 30-2021 (100ml).

Cryoprotectant Medium

Complete growth medium described above supplemented with 5% (v/v) DMSO.
Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Additional Information

Additional product and technical information can be obtained from the catalog references and the ATCC Web site at www.atcc.org, or by e-mail at tech@atcc.org.

References

(additional references may be available in the catalog description at www.atcc.org)

Sugarman BJ et al. **Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro.** Science 230: 943-945, 1985 PubMed: 86044518

Fogh J et al. **Absence of HeLa cell contamination in 169 cell lines derived from human tumors.** J. Natl. Cancer Inst. 58: 209-214, 1977 PubMed: 77097006

Lan MS et al. **Polypeptide core of a human pancreatic tumor mucin antigen.** Cancer Res. 50: 2997-3001, 1990 PubMed: 90242270

Castles CG et al. **Expression of a constitutively active estrogen receptor variant in the estrogen receptor-negative BT-20 human breast cancer cell line.** Cancer Res. 53: 5934-5939, 1993 PubMed: 94084651

Huguet EL et al. **Differential expression of human Wnt genes 2, 3, 4, and 7B in human breast cell lines and normal and**

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disease states of human breast tissue. Cancer Res. 54: 2615-2621, 1994 PubMed: 94221588

J. Natl. Cancer Inst. 21: 1131-1147, 1958

Pollack MS et al. **HLA-A, B, C and DR alloantigen expression on forty-six cultured human tumor cell lines.** J. Natl. Cancer Inst. 66: 1003-1012, 1981 PubMed: 81218998

Littlewood-Evans AJ et al. **The osteoclast-associated protease cathepsin K is expressed in human breast carcinoma.** Cancer Res. 57: 5386-5390, 1997 PubMed: 98053913

Geiger T et al. **Antitumor activity of a PKC-alpha antisense oligonucleotide in combination with standard chemotherapeutic agents against various human tumors transplanted into nude mice.** Anti-Cancer Drug Des. 13: 35-45, 1998

Hay, R. J., Caputo, J. L., and Macy, M. L., Eds. (1992), **ATCC Quality Control Methods for Cell Lines**, 2nd edition, Published by ATCC.

Caputo, J. L., **Biosafety procedures in cell culture**. J. Tissue Culture Methods 11:223-227, 1988.

Fleming, D.O., Richardson, J. H., Tulis, J.J. and Vesley, D., (1995) **Laboratory Safety: Principles and Practice**. Second edition, ASM press, Washington, DC.

Centers for Disease Control (1993), **Biosafety in Microbiological and Biomedical Laboratories** Human Health Service Publication No. (CDC) 93-8395. U.S. Dept. of Health and Human Services; 3rd Edition U.S. Government Printing Office Washington D.C.

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PFLAG A1 VECTOR MAP (BACKBONE PEGFP-C3) MARCH 2012:

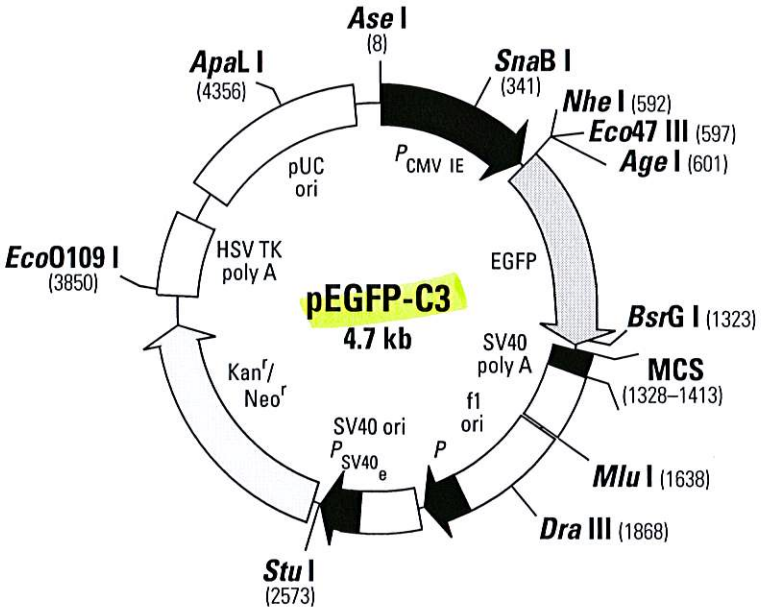
*GCTAGC***GGGATGGACTACAAGGACGACGACGACAAG***GGATCC*G*GAATTC*GG*GTCGAC*CTCGAG*GCGGCCGC*TCTAGA **pFLAG-M1**

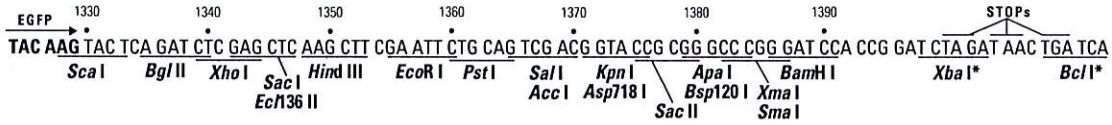
*GCTAGC***GGGATGGACTACAAGGACGACGACGACAAG***GGATCCGAATTC*GG*GTCGAC*CTCGAG*GCGGCCGC*TCTAGA **pFLAG-A1**

NheI Kozak (G/ANNATGG) FLAG BamHI EcoRI SalI XhoI NotI XbaI

pEGFP-C3 Vector Information PT3052-5

GenBank Accession #: U57607 Catalog #6082-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C3. All restriction sites shown are unique. The *Bcl* I site cannot be used for fusions since it contains an in-frame stop codon. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-C3 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-C3 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-C3 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neor), consisting of the SV40 early promoter, the neomycin/ kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-C3 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR29969; published 03 October 2002)

Use:
Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C3 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C3 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of Features:
- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP: 1327–1329
- MCS: 1328–1413
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1546–1551 & 1575–1580; mRNA 3' ends: 1584 & 1596
- f1 single-strand DNA origin: 1643–2098 (Packages the noncoding strand of EGFP)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2160–2165; –10 region: 2183–2188
  Transcription start point: 2195
- SV40 origin of replication: 2439–2574
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2272–2343 & 2344–2415
  21-bp repeats: 2419–2439, 2440–2460 & 2462–2482
  Early promoter element: 2495–2501
  Major transcription start points: 2491, 2529, 2535 & 2540
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2623–2625; stop codon: 3415–3417
  G→A mutation to remove *Pst* I site: 2805
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3151
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3653–3658 & 3666–3671
- pUC plasmid replication origin: 4002–4645

Primer Locations:
- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:
- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:
1. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

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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	Dr. Moshmi Bhattacharya
DEPARTMENT	Physiology & Pharmacology
ADDRESS	1151 Richmond St., Western University N6A 5C1
PHONE NUMBER	X82970
EMERGENCY PHONE NUMBER(S)	519-679-3135
EMAIL	Moshmi.Bhattacharya@schulich.uwo.ca

Location of experimental work to be carried out: Building(sMedical Science (MSB)_____
Room(s)__224, 231, 235____

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

FUNDING AGENCY/AGENCIES:___CIHR_____
GRANT TITLE(S): _____

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

Name	UWO E-mail Address	Date of Biosafety Training
Cynthia Pape	Cynthia.pape@schulich.uwo.ca	11 Oct.2009
Mistre Alemayehu	malemay@uwo.ca	19 Sept 2009
Jeff Law	jlaw@uwo.ca	26 May 2008
Donna Cvetkovic	dcvetco@uwo.ca	10 Feb 2011
Josh Burley		03 July 2010

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

A) The cell lines will be used to conduct various assays, that are routinely conducted in this laboratory. These include cell migration assays using transwell chambers, immunofluorescence assays to look at the localization of proteins in cells using confocal microscopy, biochemical assays to study protein expression, and motility assays to study the role of specific proteins in cell motility.

B) We have on hand biohazard agents rated Biosafety level 1 and 2, therefore all of our products will be maintained and handled at Biosafety 2 Level requirements. All students/personnel will be properly trained and supervised when handling biohazardous materials. Laboratory space/storage vessels (LN2 tank) containing biohazardous substances will be locked. All cell lines and biohazard substances will be used for research purposes alone and will be handled within a certified biological safety cabinet. All waste products will be disinfected with bleach solution or contained and autoclaved appropriately prior to disposal. Cholera toxin will be inactivated by strong acid solution (2N.HCl) or autoclaved, as recommended by the manufacturer. Pertussis toxin will be autoclaved to inactivate the compound.

Please include a one page research summary or teaching protocol.

My research program focuses on identifying and studying molecules that can be targeted in the treatment of breast cancer metastasis, the leading cause of cancer deaths . Breast cancer is the second leading cause of cancer mortality among Canadian women and it is estimated that 23,200 cases will be diagnosed and 5,300 deaths will occur in Ontario this year as a result of this devastating disease . This translates to 63 new breast cancer diagnoses and 14 breast cancer deaths each day in Ontario. ***Identifying appropriate targets for anti-metastatic intervention is therefore essential.***

We have found that G protein-coupled receptors (GPCRs), targets for 60% of all pharmaceuticals, have emerged as crucial players in tumor growth and metastasis . We found that the receptors for bio-active, blood-borne lipid lysophosphatidic acid (LPA) LPA_1 is overexpressed in breast cancer and promote metastatic spread. A key molecule in GPCR function and regulation is the GPCR adaptor protein beta-arrestin. We have recently discovered that beta-arrestins critically regulate LPA_1 receptor mediated breast cancer cell migration and invasion via the small GTPase Ral . Depletion of beta-arrestin or Ral blocks breast cancer invasiveness. ***The proposed work will investigate how beta-arrestin regulates cell migration and invasion, and the molecular determinants by which this occurs.*** Thus we ***hypothesize*** that beta-arrestins promote breast cancer cell invasiveness signaling via small GTPases Ral. The ***objectives*** of the proposed research are as follows: **(I)** To establish a role of beta-arrestin signaling in breast cancer metastasis *in vivo* and **(II)** To determine whether the expression of beta-arrestins and Ral proteins are altered in human tumor samples, and to determine their oncogenic potential.

1.0 Microorganisms

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

_____
_____
_____

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

_____
_____
_____

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.coli (DH5alpha cells)	O Yes ✓O No	O Yes ✓O No	O Yes ✓O No	3L (max 500ml/flask)	Invitrogen	✓O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

May 24/11 Per conversation with C. Pape JS

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

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes XO No		Not applicable
Rodent	O Yes XO No		
Non-human primate	O Yes XO No		
Other (specify)	O Yes X O No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	XO Yes O No	HEK 293	2	ATCC#CRL-1573
		MDA-MB-231	1	ATCC#HTB-26
		MDA-MB-231shBarr1/2	2	Stables fromHTB-26
		MDA-MB-435S	1	ATCC#HTB-129
		MDA-MB-468	1	ATCC#HTB-132
		MCF-7	1	ATCC#HTB-22
		MCF10A	1	ATCC#CRL-1-317
		MCF-12	1	
		SK-BR-3	1	ATCC#HTB-30
		Hs578T	1	ATCC#HTB-126
	Research collaborators	Hs578BST	1	ATCC#HTB-125
		OCVA429		(TSheppard)
		JEG-3	1	(LPostovit)atcc HTB-36
		JAR	1	(LPostovit)atcc HTB-144
		HTR8/SVneo		(LPostovit)
		MCF10aCA1 DCIS		(LPostovit)
		MCF10aCL1 METS		(LPostovit)
		C8161		(LPostovit)
		C81-61		(LPostovit)
		T47D	1	(LPostovit)atcc HTB-133
Rodent	O Yes XO No	RBL-1(not currently in use)	1	ATCC#CRL-1378
		GH3(proposed)	1	ATCC#CCL-82.1
Non-human primate	O Yes XO No	COS7(not currently in use)	2 (contain SV-40 viral dna sequences)	ATCC#CRL-1651
Other (specify)	O Yes XO No			

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? XO YES 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**

Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)	LLSG/UH	Not Applicable	none	Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5alpha (E.coli)	*pcdna3* *pRS* *pEYFP* *pReceiver-M13*	*Invitrogen* *Origene* *Clonetech* *GeneCopoeia*	*Numerous genes will be transfected indiviually (eg Ral, arrestin, LPA1, LPA1, Rap1); please see papers published from lab (Li et al., Molecular Cancer research 2009; Aziziyeh et al., 2009 Cellular Signalling)*	*Plasmids commercially available -- note websites below*

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

** Please attach a plasmid map.

http://products.invitrogen.com/ivgn/product/V79520?ICID=search-product

http://www.origene.com/other/products/TR20003.aspx
http://www.origene.com/assets/Documents/msds/HuSHshRNAMaterialSafetyDataSheet.pdf

http://www.clontech.com/images/pt/dis_vectors/PT3175-5.pdf

http://www.genecopoeia.com/tech/omicslink/pReceiver-M13.pdf

http://www.ncbi.nlm.nih.gov/pubmed/19609003

http://www.ncbi.nlm.nih.gov/pubmed/19306925

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O YES, complete table below XO 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.

May 24/11 per conversation with C. Pepe JS.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES n/aO NO

4.6 Will virus be infectious to humans or animals? O YES n/aO NO

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

5.0 Human Gene Therapy Trials

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

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used____mouse_____

6.3 AUS protocol # _____2008-086-06_____

6.4 Will any of the agents listed in section 4.0 be used in live animals XO YES, specify: MDA-MB-231shBarr1; 231 shBarr2; 231shBarr1&2_____ O NO

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

_____

Live cells (cell lines) will be injected into the bloodstream of live mice. The mouse body/environment is not optimal for these cells to grow, and it is expected that most of the cells will die. Those cells that do survive will form micro tumours and eventually visible tumours in lung tissue of the mice. Mice will be euthanized and lung tissues collected and fixed for further analysis.

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Will live animals be used? O YES O No

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

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O 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? X O YES O NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)__Cholera Toxin (CTX Sigma #C8052)
http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do;
Pertussis Toxin (PTX Sigma #P7208)_
http://www.sigmaaldrich.com/catalog/DisplayMSDSContent.do_____
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____CholeraToxin 250ug/kg mouse 260ug/kg i.v.; PertussisToxin - rat 114ug/kg i.v. ; mouse 127ug/kg i.v._____

8.4 How much of the toxin is handled at one time*? __CTX: 50ng ; PTX: 2ug_____

8.5 How much of the toxin is stored*? _____CTX: 1 mg ; PTX: 50ug_____

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

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

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

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

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

10.0 Plants

10.1 Do you use plants? O YES XO NO If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? XO YES, please give country of origin_USA_; Sigma-Aldrich takes care of import/export licenses for CTX & PTX; Cedarlane Labs takes care of import/export requirements to obtain cell lines thru ATCC_; most recent PHAC acknowledgement of Biosafety level 2 containment 20 May 2010__HPTA #R-06-000598_____ O NO

If no, please proceed to Section 12.0

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

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

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

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

XO YES, date of most recent biosafety inspection: _22March2010_____

O NO, please certify

O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _BIO-0122 last inspection 22 March 2010_____

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.
Cholera Toxin is inactivated by treatment with mild acid or heat, so will be inactivated by use of 1-2N HCl or by autoclaving. Pertussus toxin will be inactivated thru autoclaving

_____

_____

_____

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:

_First aid procedures would involve removal & containment of the product in question from skin/surfaces by washing with cold water to reduce absorption thru pores of the skin or use of eye wash as required: 15-20 minute wash recommended (1 eye wash is located in each of our laboratory spaces). Eye exposure would be followed up with appropriate medical/emergency room visit, as would other exposure if irritation persists. A safety shower is available and recommended for large spills. Animal experiments involving injection of cells into mice would be performed in a certified CL2 containment facility with prior ACVS approval by properly trained personnel on animals that are appropriately restrained for the procedure. Property/waste cloths would be decontaminated with bleach & or autoclaved prior to disposal after the individual affected has been tended to. _____

_____

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: May 24, 2011

15.0 Approvals

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

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____ May 2011

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: J Stanley
Date: May 07/11

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

Special Conditions of Approval:

Info on Cell Line(s)

Cell Biology

ATCC® Number:	**HTB-129™** Order this Item
Designations:	MDA-MB-435S
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	spindle shaped 
Source:	**Organ:** previously described as: mammary gland; breast **Disease:** previously described as ductal carcinoma **Derived from metastatic site:** pleural effusion
Cellular Products:	tubulin; actin
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:** 1976
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X CSF1PO: 11 D13S317: 12 D16S539: 13 D5S818: 12 D7S820: 8,10 THO1: 6,7 TPOX: 8,11 vWA: 16,18

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

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

modal number = 56; range = 55 to 62

------- Original Message --------
Subject: Re: Containment Level request: modified MDA-MB-231 cell lines
Date: Tue, 22 Sep 2009 11:45:19 -0400
From: Jennifer Stanley <jstanle2@uwo.ca>
To:genevieve_lacroix@phac-aspc.gc.ca

Hi Genevieve
Thank you for your voicemail. We will use Level 2 containment for these cells
Jennifer

Jennifer Stanley wrote:
> Hi Genevieve:
>
> I left you a voicemail. We are planning on using these under Level 2
> containment.
> Regards,
> Jennifer
>
>
>
>
> -------- Original Message --------
> Subject: Re: Containment Level request: modified MDA-MB-231 cell
> lines
> Date: Wed, 16 Sep 2009 11:37:33 -0400
> From: Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
> To: Jennifer Stanley <jstanle2@uwo.ca>
>
>
>
> Dear Jennifer,
>
> I am sorry for the delayed answer. I believe it would be easier to
> discuss this case over the phone. Please call me at your convenience.
>
> Regards
>
> Genevieve Lacroix, M.Sc.
> Senior Biosafety Officer / Inspecteur principal, biosécurité Pathogen
> Regulation Directorate (formerly Office of Laboratory Security) /
> Direction de la réglementation des agents pathogènes (anciennement le
> Bureau de sécurité des laboratoires) Public Health Agency of Canada /
> Agence de la santé publique du Canada
> 100 ch. Colonnade Rd. AL: 6201A, Ottawa, Ontario, Canada, K1A 0K9
> Tel: (613) 946-6982
> Fax: (613)941-0596
> genevieve_lacroix@phac-aspc.gc.ca
> http://www.phac-aspc.gc.ca/ols-bsl/index.html
>

>
> Jennifer Stanley <jstanle2@uwo.ca> 2009-09-14 03:59 PM
>
> To
> Geneviève Lacroix <genevieve_lacroix@phac-aspc.gc.ca>
> cc
>
> Subject
> Containment Level request: modified MDA-MB-231 cell lines
>
>
>
>
>
> Hi Genevieve
> Please let me know what containment level you would recommend for
> these modified MDA-MB-231 cell lines (knock down shRNA) B-arrestin 1
> and/or B-arrestin 2 contructs.
> See details below.
> Jennifer
>
>
> Initial investigation in our lab showed elevated endogenous levels of
> B-arrestin2 mRNA in human breast carcinoma cell lines. Transient
> transfection with shRNA constructs (commercially available)
> to B-arrestin1 (shBarr1) and/or B-arrestin2 (shBarr2) to knock-down
> (k/d) but not eliminate B-arrestin signaling showed a marked decrease
in
> the motility of the cells in response to stimulation with either 10uM
> LPA (lysophosphatidic acid) or to serum, however the efficiency of
> transfection was very low. Stable cell lines were generated using
> MDA-MB-231 parent cells electroporated with shBarr1 and/or shBarr2.
A
> control 'scrambled' line was also generated. These cell lines require
> the use of antibiotic (puromycin) to provide selective pressure for
the
> cells to maintain the transfected shRNA construct (pRS vector). The
> stable cell lines also show significant reduction in cell motility
> (2D/migration assay) as well as a decrease in the formation of
stellate
> structures (3D/invasion assay) as compared to the parent MDA-MB-231
> cells.
>
>
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>

Cell Biology

ATCC® Number:	**HTB-132™** Order this Item	Price:	**$279.00**

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

Related Links

NCBI Entrez Search
Make a Deposit
Frequently Asked Questions
Material Transfer Agreement
Technical Support
Related Cell Culture Products

Login Required

Product Information Sheet

BioProducts

Cell, microbial and molecular genomics products for the life sciences

BioServices

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

modal number = 64; range = 60 to 67.

Cell Biology

ATCC® Number:	**HTB-22™**	Order this Item	Price:	**$279.00**

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

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modal number = 82; range = 66 to 87.

Cell Biology

ATCC® Number:	**CRL-10317™** Order this Item	Price:	**$279.00**

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

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Cell Biology

ATCC® Number:	**HTB-30™** Order this Item	Price:	**$279.00**
Designations:	SK-BR-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:** mammary gland; breast **Disease:** adenocarcinoma **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.		
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:** 1970		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Tumorigenic:	Yes		
Antigen Expression:	Blood Type A; Rh+; HLA A11, Bw22(+/-), B40, B18		

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Amelogenin: X

Cell Biology

ATCC® Number:	**HTB-36™** Order this Item	Price:	**$279.00**
Designations:	JEG-3		
Depositors:	G Kohler		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial 		
Source:	**Organ:** placenta **Disease:** choriocarcinoma		
Cellular Products:	human chorionic gonadotropin (hCG), human chorionic somatomammotropin (placental lactogen); progesterone		
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 (Roche FuGENE® Transfection Reagents)		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9,11 D16S539: 13,14 D5S818: 10,11 D7S820: 10,12 THO1: 9,9.3 TPOX: 8 vWA: 16		

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Cytogenetic Analysis: This is a hypertriploid human cell line. The modal chromosome

Cell Biology

ATCC® Number:	**HTB-144™**	Order this Item	Price:	**$279.00**

Designations:	JAR
Depositors:	RA Pattillo
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** placenta **Disease:** choriocarcinoma
Cellular Products:	estrogen; progesterone; human chorionic gonadotropin (hCG); human chorionic somatomammotropin (placental lactogen); hCG production averages 22.5 ng/ml after reculturing
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.
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 7,10 D13S317: 11 D16S539: 9,10 D5S818: 10,11 D7S820: 10,11 THO1: 6,7 TPOX: 8,11 vWA: 16,18

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This is probably a pseudotriploid human cell line with the modal

Cell Biology

ATCC® Number:	**HTB-133™**	Order this Item	Price:	**$279.00**

Designations:	T-47D
Depositors:	I Keydar
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
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

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DNA Profile (STR): Amelogenin: X

Cell Biology

ATCC® Number:	**CRL-1378™** Order this Item	Price:	**$329.00**

Designations:	RBL-1
Depositors:	H Metzger, C Isersky
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	Rattus norvegicus (rat)
Morphology:	lymphoblast
Source:	**Organ:** peripheral blood **Strain:** Wistar **Disease:** leukemia **Cell Type:** basophil; chemically induced
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)
Receptors:	FcERI (Fc of IgE)
Comments:	The line exhibits various characteristics of basophil differentiation including surface receptors for IgE. It was shown to not release histamine by an IgE mediated system. PubMed: 6166481
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition or replacement of fresh medium. Start cultures at 2 X 10 exp5 viable cells/ml and maintain between 1 X 10 exp5 cells/ml and 1 X 10 exp6 /ml.. **Medium Renewal:** Add medium as cell density increases
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or

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Cell Biology

ATCC® Number:	**CCL-82.1™**	Order this Item	Price:	**$329.00**

Designations:	GH3
Depositors:	AH Tashjian
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	loosely adherent with floating clusters
Organism:	Rattus norvegicus (rat)
Morphology:	epithelial
Source:	**Organ:** pituitary **Strain:** Wistar-Furth **Disease:** tumor
Cellular Products:	prolactin; growth hormone (somatotrophin)
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:** July, 1965
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Herpes simplex virus Vesicular stomatitis virus Human poliovirus 1
Cytogenetic Analysis:	modal number = 67; range = 47 to 71. Stemline karyotype is stable with a few structural alterations. Two dicentric marker chromosomes were observed in 100% of the cells examined.
Age:	7 months
Gender:	female

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Clone GH3 was established in July, 1965 by A.H. Tashjian, Jr., et

Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$279.00**

Designations:	COS-7
Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Cercopithecus aethiops*
Morphology:	fibroblast 
Source:	**Organ:** kidney **Cell Type:** SV40 transformed
Cellular Products:	T antigen
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)
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.
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 (CO2), 5% **Temperature:** 37.0°C

Protocol:

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Cell Biology

ATCC® Number:	**CRL-1573™** Order this Item	Price:	**$279.00**

Designations:	293 [HEK-293]
Depositors:	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** embryonic kidney **Cell Type:** transformed with adenovirus 5 DNA
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) viruscide testing [92579]
Receptors:	vitronectin, expressed
Tumorigenic:	YES
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19

This is a hypotriploid human cell line. The modal chromosome

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Cell Biology

ATCC® Number:	**HTB-26™**	Order this Item	Price:	**$279.00**

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

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The cell line is aneuploid female (modal number = 64, range = 52 to

Toxin Info

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Cholera toxin
Proposed Use Dose:	0.05 µg
Proposed Storage Dose:	1000 µg
LD_{50} (species):	250 µg

Calculation:

250 µg/kg x 50 kg/person

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

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

Comments/Recommendations:

Western

TOXIN USE RISK ASSESSMENT

Name of Toxin:	Pertussis toxin
Proposed Use Dose:	2 µg
Proposed Storage Dose:	50 µg
LD_{50} (species):	114 µg

Calculation:

114 µg/kg x 50 kg/person

Dose per person based on LD_{50} in µg = 5700

LD_{50} per person with safety factor of 10 based on LD_{50} in µg = 570

Comments/Recommendations: