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:** _____

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

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

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

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-

American Type Culture Collection
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 (U.S., Canada, and Puerto Rico)
703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org

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)

Fogh, J., ed., **Human tumor cells in vitro**. New York: Plenum Press; 1975:pp. 115-159

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

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

Fogh J et al. **One hundred and twenty-seven cultured human tumor cell lines producing tumors in nude mice.** J. Natl. Cancer Inst. 59: 221-226, 1977 PubMed: 77210034

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

Hudziak RM et al. **Monoclonal antibodies directed to the Her2 re ceptor.** U.S. Pat. 5,677,171 dated Oct. 14, 1997

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

Chavany C et al. **p185erbB2 binds to GRP94 in vivo.** J. Biol. Chem. 271: 4974-4977, 1996 PubMed: 96214925

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.

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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.

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

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

*GCTAGC*GGGATGGACTACAAGGACGACGACGACAAG*GGA*[redacted]*GAA*[redacted]GG*G*[redacted]*ACC*[redacted]AG*GCGGCCGC*TCTAGA **pFLAG-A1**

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

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 GGGCCCG*AAGCTT*gagaggggcgttgtcctcccccagg