Modification Form for Permit BIO- UWO-01 74

Permit Holder: Vincent Morris

Approved Personnel (Please stroke out any personnel to be removed)	**Additional Personnel** (Please list additional personnel here)
Nicole Hague	Dr. Joaquin Madrenas
Ian MacDonald	

	Please stroke out any approved Biohazards to be removed below	**Write additional Biohazards for approval below. ***
Approved Microorganisms	E.coli - dH5 Alpha	
Approved Cells	rodent (C57B/6), rodent (mmpq), rodent (C57B1/6), mouse melanoma, B1 6F1, B16F10, MDAMB 231	Cloudman S91 mouse melanoma cells purchased from American Type Culture Collection. Noninfectious.
Approved Use of Human Source Material		
Approved GMO	pcDNA 3.1 Hygro, pcDNA 3.1 neo	
Approved use of Animals	mice	
Approved Toxin(s)		

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: Vincent Lapointe

Classification: 1

Date of Last Biohazardous Agents Registry Form: Aug 24, 2007

Date of Last Modification (if applicable): Jul 10, 2009

BioSafety Officer(s):

Chair, Biohazards Subcommittee:

Cloudman mouse melanoma cells are used as allogenic cells to investigate immunosuppression by melanoma cells.

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Cell Biology

ATCC® Number: CCL-53.1™ Order this Item

Price: $323.00

Designations: Clone M-3 [Cloudman S91 melanoma]

Depositors: G Sato

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: epithelial

Source:
Organ: skin
Strain: DBA
Disease: melanoma
Cell Type: melanocyte;

Cellular Products: melanin

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.

Virus Susceptibility: herpes simplex; vaccinia; pseudorabies; vesicular stomatitis (Indiana)

Virus Resistance: poliovirus 1

Tumorigenic: Yes

Reverse Transcript: positive

Cytogenetic Analysis: Stemline number is hypertetraploid. Karyotype is stable within stemline number. Marker chromosomes: A medium size chromosome with a submedian centromere and a smaller chromosome with a median centromere., The remaining 81 chromosomes have terminal centromeres, the first one is larger than normal. A minute chromosome was noted in 20% of the cells.

Gender: male

Comments: Clone M-3, a melanin-producing cell line was adapted to cell culture by Y. Yasumura, A.H. Tashjian and G. Sato from a Cloudman S91 melanoma in a (C X DBA) F1 male mouse obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine.
Tested and found negative for ectromelia virus (mousepox).

Propagation:

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

We will also be including the mouse cell line Cloudman S91 melanoma cells. These cells will be purchased from American Type Culture Collection and are pathogen free. We are using these cells because they are allogenic to the mice listed above and allow use to determine the immunosuppressive ability of the melanoma cells in an allogenic host.

Modification Form for Permit BIO-UWO-0174

Permit Holder: Vincent Morris

Approved Personnel
(Please stroke out any personnel to be removed)

Nicole Hague

Ian MacDonald

Additional Personnel
(Please list additional personnel here)

see e-mail attached V.

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		E.coli - dH5alpha
Approved Cells	rodent (C57B/6), rodent (mmpq), rodent (C57B1/6), mouse melanoma, B16F1, B16F10	MDAMB 231
Approved Use of Human Source Material		
Approved GMO		pcDNA3.1 Hygro, pcDNA3.1 neo
Approved use of Animals	mice	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 1

Date of last Biohazardous Agents Registry Form: Aug 24, 2007

Signature of Permit Holder: Vincent Morris

BioSafety Officer(s): JStanley July 10/09

Chair, Biohazards Subcommittee: C M. Kribs

Modification Form for Permit BIO-UWO-0174

Permit Holder: Vincent Morris

Approved Toxin(s)

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 1

Date of last Biohazardous Agents Registry Form: Aug 24, 2007

Signature of Permit Holder: Vincent Morris

BioSafety Officer(s): [illegible] July 10/09

Chair,Biohazards Subcommittee: [illegible]

Subject: Re: [Fwd: Re: MOdification form: MOrris]
From: Vincent L Morris <vmorris@uwo.ca>
Date: Thu, 25 Jun 2009 20:11:02 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

To Jennifer Stanley:

The MDAMB 231 cell line is an established breast tumor cell line. We are using it to determine how breast tumor cells spread to and grow in lymph nodes.

Thanks,
Vince

----- Original Message -----
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Thursday, June 25, 2009 3:10 pm
Subject: [Fwd: Re: MOdification form: MOrris]
To: Vincent L Morris <vmorris@uwo.ca>

> Hi Dr. Morris
> OK - cloning strains of E. coli (ie dH5 alpha)
> Can you provide some details on the use of the MDAMB 231 cell line?
> Thanks
> Jennifer
>
> -------- Original Message --------
> Subject: Re: MOdification form: MOrris
> Date: Wed, 27 May 2009 20:25:12 -0400
> From: Vincent L Morris <vmorris@uwo.ca>
> To: Jennifer Stanley <jstanle2@uwo.ca>
> References: <4A159F0C.1050800@uwo.ca>
> <fc24e515711e.4a1a5723@uwo.ca>
> <4A1A99FF.5030208@uwo.ca> <4A1BFDD1.7080800@uwo.ca>
>
>
>
> Thanks,
>
> Vince Morris
>
> ----- Original Message -----
> From: Jennifer Stanley <jstanle2@uwo.ca>
> Date: Tuesday, May 26, 2009 10:33 am
> Subject: Re: MOdification form: MOrris
> To: Vincent L Morris <vmorris@uwo.ca>
>
> > Dr. Morris
> > I will also put on it E.coli and the plasmids you use in the

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Cell Biology

ATCC® Number: HTB-26™ Order this Item

Designations: MDA-MB-231

Biosafety Level: 1

Medium & Serum: See Propagation

Organism: *Homo sapiens* (human)

Price: $256.00

Depositors: R Cailleau

Shipped: frozen

Growth Properties: adherent

Morphology: epithelial

PHOTO

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.

Related Cell Culture Products

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Receptors: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Yes

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes:
AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Use of MDAMB 231 cells by Vincent L. Morris

We are investigating the spread of mammary tumor cells to lymph nodes and other tissues and how these cells can colonize lymph nodes and other organs. Metastasis of mammary tumor cells and their ability to evade the body's immune system is the main reason mammary tumor cells are so deadly. If we can determine the factors affecting the spread of mammary tumor cells and how they evade the bodies immune defenses, we can reduce the number of deaths from this form of cancer. We wil also compare the metastasis of mammary tumor and melanoma cells to assist in determining how both types of tumor cells spread.

Vincent Morris

1.15. Will genetic modifications be made to the microorganisms or biological agents described in 1.1.?	☒ Yes ☐ No	☒ Yes ☐ No	☒ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
If 'Yes' to 1.15. Complete Table 1.15.1. for Plasmids and/or 1.15.2. for Viral Vectors					
1.15.1. PLASMIDS					
Bacteria Used for Cloning	E. coli				
Plasmid(s)	pcDNA3.1Hygro; pcDNA3.1 neo				
Plasmid Source	Invitrogen				
Gene Transfected	Tdtomato;beta galactosidase;mko2hcdf1;mAGhGem				
Describe Resulting Change	marks cells with a staining or fluoresecent tag				
Is this expected to increase the invasiveness, toxicity, or tumorgenicity of the agent in the animal?	☐ Yes ☒ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No
1.15.2. VIRAL VECTORS					
Virus Used for Transfection					
Vector(s)					
Vector Source					
Gene Transfected					
Describe Resulting Change					
Is this expected to increase the invasiveness, toxicity, or tumorgenicity of the agent in the animal?	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No

9.1.2. Risk Control Measures – List the control measures required to eliminate or further minimize the risk.

ACTIVITY/HAZARD	RISK CONTROL MEASURES/ACTION
1.2.1. What will you do it someone is bitten or scratched?	injected cells or microspheres pose no added threat to humans, Follow workplace health procedure for bites and scratches (see attached)
1.2.2. How are contaminated materials to be treated prior to disposal?	decontamination by incineration, chemical disinfection or autoclaving
1.2.3. How are the contaminated carcasses to be disposed of?	incineration
1.2.4 List all **preventative measures** to be taken to minimize the risk of exposure to RESEARCH STAFF handling the material	☒ Safety Glasses ☒ Gloves ☒ Lab coat or equivalent ☒ Mix solutions and handle agent(s) in the fumehood ☐ Contact Workplace Health regarding the medical surveillance required to handle these agents. ☒ N95 Fit-tested Respirator, *specify type*: ☐ Other, *please specify*:
1.2.5. List all **preventative**	☒ Safety Glasses

Subject: Re: MOdification form: MOrris
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 26 May 2009 10:33:53 -0400
To: Vincent L Morris <vmorris@uwo.ca>

Dr. Morris
I will also put on it E.coli and the plasmids you use in the animal protocol, just for the sake of completion (no safety concerns). I assume that you use cloning E.coli (ie dH5 alpha or something like that)?
Jennifer

Jennifer Stanley wrote:
> Got it - thanks!
>
> Vincent L Morris wrote:
> > To Jennifer:
> >
> > I have faxed a copy of the modification to you. I hope you received it.
> >
> > Thanks,
> > Vince
> >
> > ----- Original Message -----
> > From: Jennifer Stanley <jstanle2@uwo.ca>
> > Date: Thursday, May 21, 2009 2:35 pm
> > Subject: MOdification form: MOrris
> > To: Vincent L Morris <vmorris@uwo.ca>
> >
> > > Hi Dr. Morris
> > > Please update this paperwork as soon as possible - your AUS
> > > protocol can
> > > not be approved without it.
> > > Thanks
> > > Jennifer
> > >
> > > -------- Original Message --------
> > > Subject: MOdification form: MOrris
> > > Date: Tue, 12 May 2009 13:57:46 -0400
> > > From: Jennifer Stanley <jstanle2@uwo.ca>
> > > To: Vincent L Morris <vmorris@uwo.ca>
> > >
> > >
> > >
> > > Dr. Morris:
> > > I received an AUS protocol for you which uses the cell line MDA
> > > MB231 cell line. Please complete the attached Modification
> > > form to add this to your existing permit. For more
> > > information, please see:
> > >
> > http://www.uwo.ca/humanresources/docandform/forms/ohs/bio_modification_info.pdf
> > > Thanks!
> > > Jennifer
> > >
> > >
> > >
> > >
> > >

Summary of Approvals for Permit BIO-UWO-0174

Permit Holder: Vincent Morris

Approved Personnel (Please stroke out any personnel to be removed

Additional Personnel

Dr. Ian McDonald
Nicole Hague

	Please stroke out any approved Biohazards* to be removed below	Write additional Biohazards for approval below.
Approved Microorganisms*		
Approved Cells*	rodent (C57B/6), rodent (mmpq)	Rodent (C57B1/6) mouse melanoma B16F1 and B16F10
Approved Use of Human Source Material*		
Approved GMO*		
Approved use of Animals*	mice	
Approved Toxin(s)*		

Date of last Biohazardous Agents Registry Form Aug 24, 2007

Signature of Permit Holder: Vincent Morris

BioSafety Officer(s): A Stanley March 28/07

Chair, Biohazards Subcommittee:
G M Kidder 28 March 2008

B10-uwo-0174

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Vincent L. Morris
SIGNATURE Vincent Morris
DEPARTMENT Microbiology & Immunology
ADDRESS 3014DSB
PHONE NUMBER 83452
EMAIL vmorris@uwo.ca

Location of experimental work to be carried out: Building(s) MSA Room(s) 312A
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
Role of Matrix Metalloproteinases in Cell Movement
Coordinated regulation of epidermal growth factor- and integrin-stimulated migration of primary keratinocytes)a

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES NSERC

Names of all personnel working under Principal Investigators supervision in this location:
i) None
ii)
iii)
iv)
v)

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☐ YES ☒ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required. 1 2 3

1.4 Source of microrganism(s) or biological agent(s)? _____

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	☐ Yes ☐ No	
Rodent	☒ Yes ☐ No	C57Bl6 of MMP9- 2day old pups
Non-human primate	☐ Yes ☐ No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	☐ Yes ☐ No		
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.4 For above named cell type(s) circle HC or CFIA containment level required 1 2 3

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

3.0 Use of Human Source Materials

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

3.2 Indicate if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human organs (unpreserved) ☐ YES ☐ NO If YES, Specify_____
- Human tissues (unpreserved) ☐ YES ☐ NO If YES, Specify_____

3.3 Is human source known to be infected with and infectious agent ☐ YES ☐ NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

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 sequences from the following be involved:

- HIV ☐ YES ☐ NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐ YES ☐ NO
if YES specify_____
- Other human or animal pathogen and or their toxins ☐ YES ☐ NO
if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☐ YES ☐ NO If YES specify_____
- Known oncogenes ☐ YES ☐ NO If YES specify_____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES ☐ NO
If YES name virus_____.

4.5 List specific vector(s) to be used:_____

4.6 Will virus be replication defective ☐ YES ☐ NO

4.7 Will virus be infectious to humans or animals ☐ YES ☐ NO

4.8 Will this be expected to increase the Containment Level required ☐ YES ☐ NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? ☐ YES ☒ NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? ☐ YES ☐ NO

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? ☐ YES ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☐ YES ☒ NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used mice 9l

6.3 AUS protocol # 2005·017·04 renewed May 1/07 9l.

6.4 If using murine cell lines, have they been tested for murine pathogens? ☐ YES ☐ NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used:

- Pound source dogs ☐ YES ☒ NO
- Pound source cats ☐ YES ☒ NO
- Sheep or goats ☐ YES ☒ NO
- Non- Human Primates ☐ YES ☒ NO If YES specify species _____
- Wild caught animals ☐ YES ☒ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin _____

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

9.0 Import Requirements

9.1 Will the agent be imported? ☐ YES ☒ NO
If no, please proceed to Section 10.0
If yes, country of origin_____

9.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ☐ YES ☐ NO

9.4 Has the import permit been sent to OHS? ☐ YES ☐ NO
If yes, Permit # _____

10.0 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

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

SIGNATURE Vincent Lomas

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. (1) 2 3

11.2 Has the facility been certified by OHS for this level of containment? ☒ YES ☐ NO
Job Watson

11.3 If yes, please give the date and permit number: N/A

12.0 Approvals

UWO Biohazard Subcommittee:

Signature G M Kidder Date 29 Aug. '07

Safety Officer for Institution where experiments will take place

Signature J Stanley Date Aug 24/07

Safety Officer for University of Western Ontario (if different than above)

Signature _____ Date _____

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