Attention: Jennifer Stanley

Modification Form for Permit BIO-UWO-0158

Permit Holder: Jim Karagiannis

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

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	Schizosaccharomyces pombe, E. coli (DH5 alpha)	
Approved Cells		
Approved Use of Human Source Material		
Approved GMO		# pCL-MFG-BRCA1 pFAGa-PA-GFP-KanMX6
Approved use of Animals		
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: May 25, 2007

Signature of Permit Holder: J. Karagiannis

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Hi Jennifer,

For plasmid pCL-MFG-BRCA1: The plasmid will be used for cross-species expression in the fission yeast, Schizosaccharomyces pombe.

For plasmid pFA6A-GFP-KanMX6: The plasmid will be used to monitor intracellular localization of proteins in the fission yeast, Schizosaccharomyces pombe.

Best,
Jim.

Plasmid 12341: pCL-MFG-BRCA1

Gene/insert name:	BRCA1
Insert size (bp):	Unknown
Gene/insert aliases:	BRCA1, IRIS, PSCP, BRCAI, BRCC1, RNF53
Species of gene(s):	H. sapiens (human)
Vector backbone:	pCLMFG (Search Vector Database)
Backbone manufacturer:	Verma Lab
Type of vector:	Mammalian expression,Retroviral
Backbone size (bp):	Unknown
Cloning site 5':	NcoI (partial digest)
Site destroyed during cloning:	No
Cloning site 3':	XbaI/BamHI
Site destroyed during cloning:	Yes
5' Sequencing primer:	LXSN primer (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Inder M Verma
Terms and Licenses:	MTA

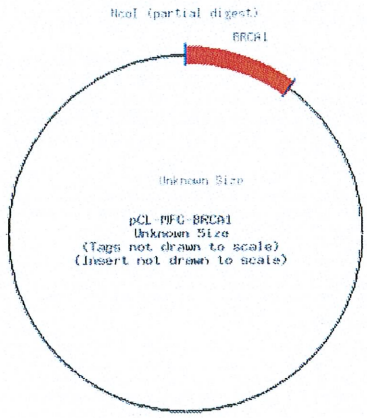
Plasmid Links
Sequence
Related Plasmids
From this article
BRCA1 plasmids
Inder M Verma Lab Plasmids
Other Links
NCBI: BRCA1
BRCA1 antibodies

This is commonly requested with
pBABEpuro HA Brca1

Comments: Full-length BRCA1 cDNA was lifted from this construct as a NcoI (partial digest)-XbaI/blunted fragment and inserted into NcoI (partial digest)-cut and BamHI-cut/blunted pCL-MFG retroviral vector.

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

Click on map to enlarge



Article: BRCA1 is a cell cycle-regulated nuclear phosphoprotein. Ruffner H et al. (Proc Natl Acad Sci U S A. 1997 Jul 8. 94(14):7138-43. Pubmed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 12341" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

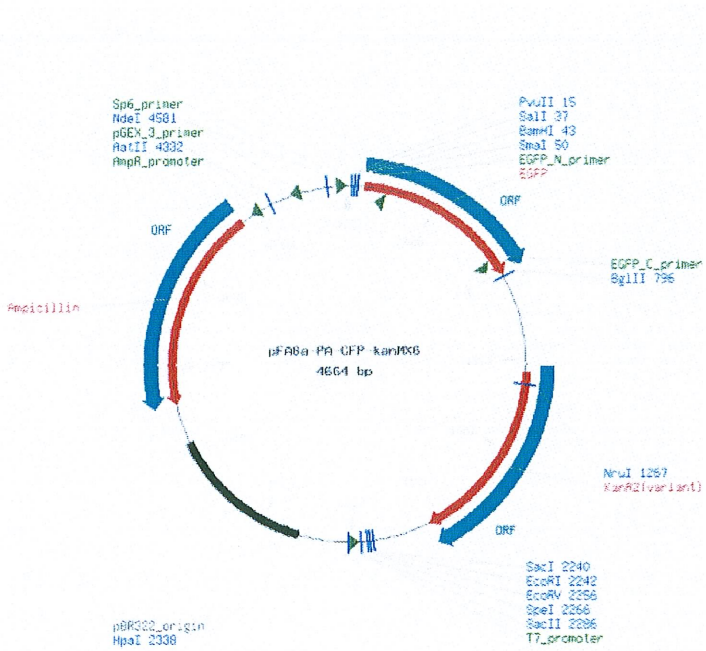
Plasmid 18820: pFA6a-PA-GFP-kanMX6

Gene/insert name:	Photoactivatable Green Fluorescent Protein
Alternative names:	PA-GFP Photoactivatable GFP
Insert size (bp):	720
Species of gene(s):	Aequorea victoria
Vector backbone:	pFA6a-kanMX6 (Search Vector Database)
Type of vector:	Yeast tagging
Backbone size (bp):	3944
Cloning site 5':	BamHI
Site destroyed during cloning:	No
Cloning site 3':	BglII
Site destroyed during cloning:	No
5' Sequencing primer:	CGATTTAGGTGACACTAT (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	Unknown
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Gentamicin
If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid:	Patricia Wadsworth, University of Massachusetts Amherst (pIREShyg-PA-GFP-alpha Tubulin)
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Wei-Lih Lee
Terms and Licenses:	MTA

Comments: U.S. Tulu, et al. (2003). "Peripheral, Non-Centrosome-Associated Microtubules Contribute to Spindle Formation in Centrosome-Containing Cells" Curr Biol 13: 1894-99.

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

Click on map to enlarge



Selected features

EGFP_N_primer	142 - 121
EGFP	76 - 792
ORF frame 1	76 - 795
EGFP_C_primer	729 - 750
ORF frame 3	1185 - 1994
KanR2(variant)	1215 - 1994
T7_promoter	2320 - 2302
pBR322_origin	3182 - 2563
ORF frame 1	4197 - 3337
Ampicillin	4197 - 3337
AmpR_promoter	4267 - 4239
pGEX_3_primer	4448 - 4426
Sp6_primer	4648 - 1

Unique restriction sites

PvuII	15
SalI	37
BamHI	43
SmaI	50
BglII	796
NruI	1267
SacI	2240
EcoRI	2242
EcoRV	2256
SpeI	2266
SacII	2286
HpaI	2338
AatII	4332
NdeI	4581

Please acknowledge the principal investigator if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 18820" in your Materials and Methods section.
This information allows Addgene to create a link from the plasmid page to your publication.

B10-UWO-0158

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biosafety Committee: October 25, 2004

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, 2nd edition, 1996, 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 Jim Karagiannis
SIGNATURE J. Karagiannis
DEPARTMENT Biology
ADDRESS Staging 109
PHONE NUMBER x8079
EMAIL jkaragia@uwo.ca

Location of experimental work to be carried out: Building(s) Staging Room(s) 109
*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):
1. Molecular Genetic Analysis of Regulatory Networks Governing Cytokinesis
2. Tetrad Dissection Microscope System for Genetic Analysis

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED.

FUNDING AGENCY/AGENCIES NSERC

Names of all personnel working under Principal Investigators supervision in this location:
i) It is expected that two graduate students will begin working Sept. 2007.
ii) Selection of individuals currently in progress.
iii)
iv)
v)

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms? ☒ YES ☐ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Microorganism	Is the microorganism known to be a human pathogen? YES/NO	Is the microorganism known to be an animal pathogen? YES/NO	Is the microorganism known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?
Schizosaccharomyces pombe	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	1 L
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
Escherichia coli O45a	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	1 L
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

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

1.4 Source of microorganism? ATCC

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 cells that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Established or Primary *
Human	☐ Yes ☐ No	☐ Established ☐ Primary
Rodent	☐ Yes ☐ No	☐ Established ☐ Primary
Non-human primate	☐ Yes ☐ No	☐ Established ☐ Primary
Other (specify)		

* i.e. derived from fresh tissue

2.3 Supplier of primary cell culture tissue _____

2.4 List specific cell lines to be used and source/supplier:_____

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

3.0 Use of Human Source Materials

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

3.2 Indicate 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 organism or cell line ? ☒ YES ☐ NO * see below
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 N/A

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

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

* I am using S. pombe as a model organism to study genetic networks governing cytokinesis. Genetic modifications will comprise deletion of genes required for cytokinesis.

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_____

6.3 AUS protocol # _____

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 _____

col # _____

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 J. Kanygin

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 + pending

11.3 If yes, please give the date and permit number:_____

12.0 Approvals

UWO Biohazard Subcommittee

Signature G.M. Golder Date 25 May '07

Safety Officer for Institution where experiments will take place

Signature Jennifer Stanley Date May 25/07

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

Signature_____ Date_____