Modification Form for Permit BIO-UWO-0103

Permit Holder: Gary Shaw

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

Brian Dempsey

Don Spratt

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	E.coli BL21	
Approved Cells	BL21 (lambda DE3)	
Approved Use of Human Source Material		
Approved GMO	pET, pGEX	pCDNA pDNR pDON
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.*

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: _____

Classification: 1

Date of Last Biohazardous Agents Registry Form: May 29, 2007

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Plasmids will be used for protein expression.

Clone: HsCD00004370

Clone ID:	HsCD00004370	Type:	cDNA
Is Verified:	Y	Verification Method:	Sequence Verification
Status:	AVAILABLE	Distribution:	No restriction
Source:	HIP	Special MTA:	
Description:			
Comments:			
Map:	pDNR-Dual_with_human_insert.pdf		

Related Identifiers:

Original Clone ID	FLH013476.01X
HIP Clone ID	13476
GI	61361493
GenBank Accession	AY890114
HIP Master Clone ID	27675

Property:

Collection	Breast Cancer 1000

Insert Information:

Insert	Size (bp)	Species	Mutation	Discrepancy	Format	Tissue Source	Species Specific ID	Gene Symbol	Gene Name	Target Genbank	Keyword
1	282	Homo sapiens	No	No	CLOSED	MGC template	6279	S100A8	S100 calcium binding protein A8 (calgranulin A)	BC005928	

Insert Sequence:

Insert: 1

```
ATGTTGACCGAGCTGGAGAAAGCCTTGAACTCTATCATCGACGTCTACCACAAGTACTCC
CTGATAAAGGGGAATTTCCATGCCGTCTACAGGGATGACCTGAAGAAATTGCTAGAGACC
GAGTGTCCTCAGTATATCAGGAAAAAGGGTGCAGACGTCTGGTTCAAAGAGTTGGATATC
AACACTGATGGTGCAGTTAACTTCCAGGAGTTCCTCATTCTGGTGATAAAGATGGGCGTG
GCAGCCCACAAAAAAAGCCATGAAGAAAGCCACAAAGAGTAG
```

Insert Property: Insert 1

Type	Value	Extra Information
End on reference sequence	337	
Start on reference sequence	56	

Vector Information:

Vector Name:	pDNR-Dual	Size (bp):	4938
Type:	bacterial plasmid	Form:	dsDNA
Description:	Recombinational donor/master vector with 6xHN tag ORF, T7 and M13 primer sites; ampicillin resistance; restriction enzyme cloning (into) and recombinational cloning (from).		
Properties:	Creator, donor (entry), loxP, multiple cloning site, recombinational cloning, with tag/fusion/marker		
Comments:	The position of features were detemined for the empty form of the vector, which is described in detail on the BD/Clontech website. Subcloning into the MCS was performed using the infusion reaction strategy and some restriction sites in the MCS may be lost after an insert is added.		
Map:	pDNR-Dual.pdf		

Sequence: pDNR-Dual_FASTA.txt

Host Information:

Host Strain	Is Used In Distribution	Description
DH5-alpha T1 phage resistant	Y	

Antibiotic Selections:

Host Type	Marker
bacterial	ampicillin

Recommended Growth Condition:

Host Type	Selection Condition	Growth Condition	Comments
bacterial	100 ug/mL ampicillin	Growth with the single antibiotic in LB at 37 degrees is recommended.	Commonly used conditions for ampicillin resistant plasmid clones.

Authors:

Author Name	Author Type
HIP	Academic Institute

Publications:

PMID	Title
16512675	Functional proteomics approach to investigate the biological activities of cDNAs implicated in breast cancer.

Clone: HsCD00005454

Clone ID:	HsCD00005454	Type:	cDNA
Is Verified:	Y	Verification Method:	Sequence Verification
Status:	AVAILABLE	Distribution:	No restriction
Source:	HIP	Special MTA:	
Description:			
Comments:			
Map:			

Related Identifiers:

HIP Clone ID	131119
Original Clone ID	FLH131119.01X
GI	60822416
GenBank Accession	AY893569
HIP Master Clone ID	107276

Property:

Collection	Breast Cancer 1000

Insert Information:

Insert	Size (bp)	Species	Mutation	Discrepancy	Format	Tissue Source	Species Specific ID	Gene Symbol	Gene Name	Target Genbank	Keyword
1	345	Homo sapiens	No	No	CLOSED	1st strand cDNA from placenta and brain	6280	S100A9	S100 calcium binding protein A9 (calgranulin B)	X06233	

Insert Sequence:

Insert: 1

```
ATGACTTGCAAAATGTCGCAGCTGGAACGCAACATAGAGACCATCATCAACACCTTCCAC
CAATACTCTGTGAAGCTGGGGCACCCAGACACCCTGAACCAGGGGGAATTCAAAGAGCTG
GTGCGAAAAGATCTGCAAAATTTTCTCAAGAAGGAGAATAAGAATGAAAAGGTCATAGAA
CACATCATGGAGGACCTGGACACAAATGCAGACAAGCAGCTGAGCTTCGAGGAGTTCATC
ATGCTGATGGCGAGGCTAACCTGGGCCTCCCACGAGAAGATGCACGAGGGTGACGAGGGC
CCTGGCCACCACCATAAGCCAGGCCTCGGGGAGGGCACCCCCTAA
```

Insert Property: Insert 1

Type	Value	Extra Information
End on reference sequence	389	
Start on reference sequence	45	

Vector Information:

Vector Name:	pDONR201	Size (bp):	4470
Type:	bacterial plasmid	Form:	dsDNA
Description:	Recombinational donor/master vector; kanamycin resistance; recombinational cloning.		
Properties:	Gateway, donor (entry), recombinational cloning		
Comments:	The position of features were determined for the unrecombined (empty) form of the vector, which is described in detail on the Invitrogen website.		

Map: pdonr201_pdonr207_map.pdf
Sequence: pDONR201_FASTA.txt

Host Information:

Host Strain	Is Used In Distribution	Description
DH5-alpha T1 phage resistant	Y	

Antibiotic Selections:

Host Type	Marker
bacterial	kanamycin

Recommended Growth Condition:

Host Type	Selection Condition	Growth Condition	Comments
bacterial	50ug/mL kanamycin	Growth with the single antibiotic in LB at 37 degrees is recommended.	Conditions for Gateway-type vectors in recombined (with insert) form and other kanamycin resistant vectors.

Authors:

Author Name	Author Type
HIP	Academic Institute

Publications:

PMID	Title

addgene

Plasmid 20717: pCDNA3-myc3-ROC1

Price: $65.00

RING finger protein 1 (ROC1)
ROC1, RNF75, RBX1
RBX1 ring-box 1
Regulator of Cullins 1
323
AF142059
RBX1, ROC1, RNF75, MGC1481, MGC13357, BA554C12.1
H. sapiens (human)
myc3
N terminal on backbone
pcDNA3
(Search Vector Database)
Invitrogen
Mammalian expression
4620
KpnI
No
XhoI
No
T7 (List of Sequencing Primers)
SP6
Ampicillin
High Copy
Yes
G418
View sequence
View map
DH5a
Yue Xiong
MTA

Author's map
Sequence
Reviews (0)

From this article
RBX1 plasmids
Yue Xiong Lab Plasmids

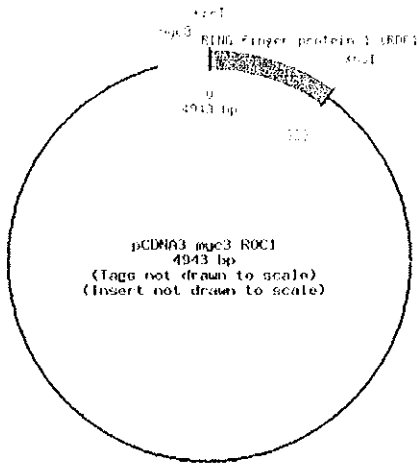
AF142059
NCBI: RBX1
RBX1 antibodies

pcDNA3-HA2-CUL4A
pcDNA3-FLAG-DDB1
pcDNA3-HA-ROC2

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

Click on map to enlarge

Protein expression.



DDB1 functions as a linker to recruit receptor WD40 proteins to CUL4-ROC1 ubiquitin ligases. He YJ et al. (Genes Dev. 2006 Nov 1. 20(21):2949-54. 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 20717" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Welcome To PlasmID

Protein Structure Initiative - Material Repository (PSI-MR)

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- Vector for Distribution

Home > My Account > Order History

Order History

View the order history and detailed order information

Order ID: 3478

1	HsCD00004370	cDNA	6279	S100A8	S100 calcium binding protein A8 (calgranulin A)	BC005928	No/No	CLOSED	pDNR-Dual	bacterial: ampicillin;
2	HsCD00005454	cDNA	6280	S100A9	S100 calcium binding protein A9 (calgranulin B)	X06233	No/No	CLOSED	pDONR201	bacterial: kanamycin;

Download Clone List

Protein expression.

BIO-UWO-0103.

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 Dr. Gary Shaw
SIGNATURE
DEPARTMENT Biochemistry
ADDRESS Medical Sciences Bldg Rm 306
PHONE NUMBER 519-661-4021
EMAIL gshaw1@uwo.ca

Location of experimental work to be carried out: Building(s) Medical Sciences Room(s) 312
*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):
Folding and Interactions of S100 Proteins
Protein Interactions in Degradation and Parkinson's Disease
Structure and Mechanism of Class II E2 Enzymes in Ubiquitylation

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

FUNDING AGENCY/AGENCIES CIHR, NCIC

Names of all personnel working under Principal Investigators supervision in this location:
i) Kathy Barber
ii) Steven Beasley
iii) Liliana Santamaria-Kisiel
iv) Nicole Marlatt
v) Ben Cook
vi) Stephanie Serniwka
vii) Susan Safadi
viii) Ventzi Hristova
ix) Atoosa Rezvanpour

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms? X 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?
E. coli - BL21	Yes X No	Yes X No	Yes X No	2 Litres
	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) circle HC or CFIA Containment Level required (1) 2 3

1.4 Source of microorganism? Commercial - Invitrogen

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? X 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 X No	Established Primary
Rodent	Yes X No	Established Primary
Non-human primate	Yes X No	Established Primary
Other (specify)	Bacteria	

* 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: BL21(2DE3) - Invitrogen

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 X 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 ? X YES NO
If no, please proceed to Section 5.0

4.2 Will genetic sequences from the following be involved:

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

4.3 Will intact genetic sequences be used from

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

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

4.5 List specific vector(s) to be used: pET, pGEX

4.6 Will virus be replication defective YES X NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES X 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 X 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 X NO
- Pound source cats YES X NO
- Sheep or goats YES X NO
- Non- Human Primates YES X NO If YES specify species _____
- Wild caught animals YES X NO If YES specify species _____

col # _____

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? YES X 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 X 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 _____

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? X YES ☐ NO

11.3 If yes, please give the date and permit number: Oct. 1, 2003 BIO-UWO-0103

12.0 Approvals

UWO Biohazard Subcommittee

Signature G M. Kidder Date 29 May '07

Safety Officer for Institution where experiments will take place

Signature D Stanley Date May 25/07

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

Signature_____ Date_____

Folding and Interactions of S100 Proteins

S100B and S100A11 are dimeric, *EF-hand* calcium binding proteins that undergo calcium-induced conformational changes, interact with specific target proteins and control specific biological activities. Calcium binding to S100B leads to modulation of the assembly and organization of cytoskeletal proteins such as AHNAK and IQGAP1 through interaction with these proteins. These effects are significantly enhanced by zinc binding to S100B. S100A11 undergoes a calcium-sensitive interaction with annexin I, thought to promote membrane aggregation. In the past five years, 3D structures of S100 proteins including several completed in our lab have revealed: (1) calcium binding results in a reorientation of helix III in calcium-binding site II, (2) target peptides bind to S100 proteins in different orientations, and (3) the peptides bound to S100s occupy only a portion of the exposed hydrophobic surface. The structures have raised many other questions that impact their biological activity including: (1) how does zinc binding modify and enhance target protein binding, (2) do multiple target binding sites exist, (3) how is S100 calcium affinity moderated, (4) how is the apo-state conformation controlled, (5) are there other biological targets for S100A11 and, (6) does the folding pathway for the S100s provide clues for calcium and target protein regulation. *To address these questions, we will use existing and new S100 structures to show how S100B and S100A11 fold, are activated and recognize their biological targets.*

The **hypotheses** to be tested are:

(i) a complex protein recognition site exists on S100B regulated by both Ca^{2+} and Zn^{2+} binding,
(ii) dimer formation of S100B moderates its Ca^{2+} affinity,
(iii) the conformation of site II is controlled by interactions in helix III and the linker,
(iv) S100A11 has multiple target proteins containing a common VXEXL motif,
(v) a folding intermediate contributes to S100B target protein recognition.

The **specific aims** and **research plan** are:

(i) Determine how <u>both</u> Ca^{2+} and Zn^{2+} regulate the structure of S100B upon binding of the consensus peptide TRTK-12, and domains from the Ca^{2+} and Zn^{2+} sensitive proteins AHNAK and IQGAP1.
(ii) Show that a monomeric S100B protein has a higher affinity for Ca^{2+}. Site directed mutagenesis will be used to generate S100B proteins modified at the dimer interface. The impact of these substitutions on oligomerization, Ca^{2+} affinity and 3D structure will be examined.
(iii) Design a constitutively active apo-S100A11 protein. The 3D structure of apo-S100A10, which exists in a "calcium-ready" conformation in the absence of calcium, will be used as a template to design an S100A11 protein that possesses a calcium-bound conformation in its apo state. Site directed mutagenesis of key residues in helix III, the linker and site I of apo-S100A11, in combination with calcium affinity and NMR residual dipolar coupling experiments, will assess how these regions control the conformation of apo-S100A11.
(iv) Determine the structure and specificity of the newly identified target protein, annexin A6 for S100A11 using NMR spectroscopy and competition experiments with annexin I.
(v) Establish a protein folding pathway for S100B. Folding/unfolding of single Trp mutants will be studied by native state hydrogen exchange NMR experiments and fluorescence spectroscopy to determine the presence and stability of intermediate folded states.

This work will provide **significant findings** including:

(i) the first structural details that Zn^{2+} enhances calcium-dependent protein interactions with S100B,
(ii) the dependence of calcium binding affinity on S100 dimerization leading to a structural rationale for the signalling properties of the S100s,
(iii) development of a calcium-insensitive S100A11 protein to be used for *in vivo* experiments,
(iv) identification of a common annexin recognition site for S100A11 leading to a new mode of function for membrane aggregation and,
(v) evidence that apo- and Ca-S100B each exist in a range of conformations important for calcium and target protein binding.