Modification Form for Permit BIO-UWO-0103

Permit Holder: Gary Shaw

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

Steven Beasley

Kathryn Barber

~~Stephanie Berniwka~~

~~Nicole Mallatt~~

~~Ventzislava Hristova~~

Chee Wang Ng

Ben Cook

Chantal Forristal

Atoosa Rezvanpour

Mariena Silvestry

Brian Dempsey

Don Spratt

Additional Personnel
(Please list additional personnel here)

Jane Bai

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E.coli BL21	
Approved Primary and Established Cells		
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	pET, pcDNA, pGEX, pDNR, pJexpress	pSKDuet01 pSzBAD1PG pSKBAD2
Approved Use of Animals		

Approved Biological 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 STORED, USED AND DISPOSED OF..*

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: David Litchfield (for Gary Shaw)

Current Classification: 1 **Containment Level for Added Biohazards:** 1

Date of Last Biohazardous Agents Registry Form: Apr 9, 2010

Date of Last Modification (if applicable):

BioSafety Officer(s):

Chair, Biohazards Subcommittee: **Date:**

Plasmids will be used for protein expression.
-stored, used and disposed of as for other approved Plasmids/Vectors on this permit.

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pSKDuet01
Plasmid 12172

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Price: $55.00

Plasmid Links
Sequence
Reviews (0)
Related Plasmids
From this article
Hideo Iwai Lab Plasmids

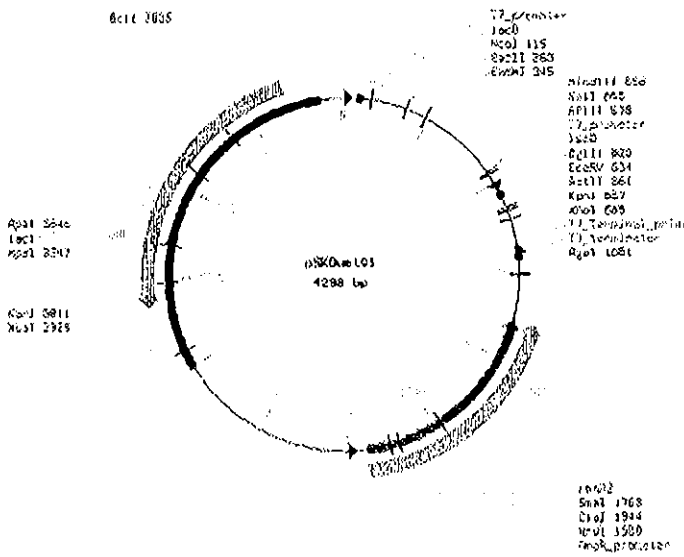
This is commonly requested with
pSKBA02

Plasmid 12172: pSKDuet01

Gene/insert name:	NpuDnaE
Alternative names:	DnaE N-intein intein
Insert size (bp):	306
Species of gene(s):	Nostoc punctiforme
Fusion proteins or tags:	His-GB1
Terminal:	N terminal on backbone
Vector backbone:	pRSFDuet (Search Vector Database)
Backbone manufacturer:	Novagen
Type of vector:	Bacterial expression
Backbone size (bp):	3757
Cloning site 5':	BamHI
Site destroyed during cloning:	No
Cloning site 3':	HindIII
Site destroyed during cloning:	No
5' Sequencing primer:	Duet-MCS1-fw (List of Sequencing Primers)
Bacteria resistance:	Kanamycin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Hideo Iwai
Terms and Licenses:	MTA

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

Click on map to enlarge



Selected features		
T7_promoter	30 - 48	→
lacO	48 - 75	→
T7_promoter	729 - 747	→
lacO	747 - 774	→
T7_Terminal_primer	981 - 963	←
T7_terminator	982 - 1017	→
ORF frame 3	2069 - 1254	←
KanR2	2069 - 1254	←
AmpR_promoter	2139 - 2111	←
lacI	4213 - 2858	←
ORF frame 2	4081 - 3122	←

Unique restriction sites	
NcoI	115
SacII	253
BamHI	345
HindIII	658
NotI	665
AflII	678
BglII	820
EcoRV	834
AatII	861
KpnI	867
XhoI	869
AgeI	1081
SmaI	1763
ClaI	1944
NruI	1980
XbaI	2929
NarI	3211
HpaI	3347
ApaI	3646
BclI	3835

Article: Highly efficient protein trans-splicing by a naturally split DnaE intein from Nostoc punctiforme. Iwai H et al. (FEBS Lett. 2006 Mar 20; 580(7):1853-8. PubMed)

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

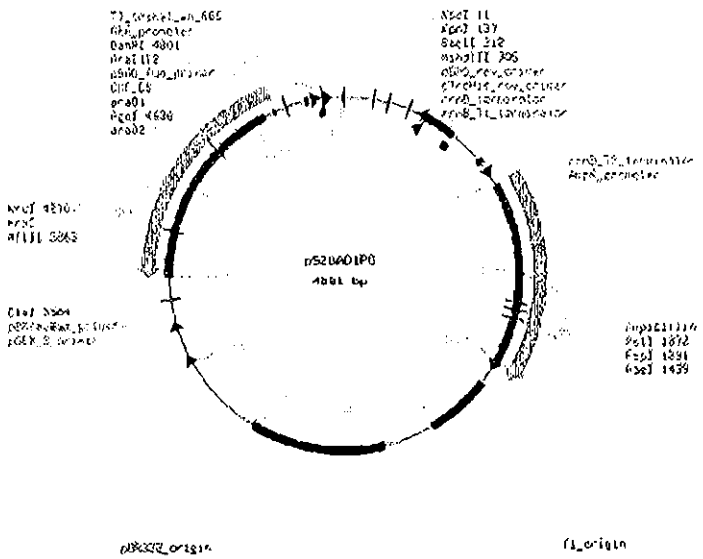
Also, please include the text "Addgene plasmid 12172" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Plasmid 11964: pSZBAD1PG

Gene/insert name:	SspDnaEC Intein
Insert size (bp):	125
Species of gene(s):	Synechocystis sp. PCC6803
Fusion proteins or tags:	GB1
Terminal:	C terminal on backbone
Vector backbone:	pBAD (Search Vector Database)
Backbone manufacturer:	invitrogen
Type of vector:	Bacterial expression
Backbone size (bp):	4766
Cloning site 5':	NdeI
Site destroyed during cloning:	No
Cloning site 3':	KpnI
Site destroyed during cloning:	No
5' Sequencing primer:	pBAD (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	Low Copy
Grow in standard E. coli @ 37C:	Yes
If you did not originally clone this gene, from whom and where did you receive the plasmid used to derive this plasmid:	From Prof. Henry Paulus, Boston Biomedical Research Institute.
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Hideo Iwai
Terms and Licenses:	MTA

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

Click on map to enlarge



Selected features		
pBAD_rev_primer	360 - 343	
pTrcHis_rev_primer	360 - 343	
rrnB_terminator	393 - 549	
rrnB_T1_terminator	516 - 656	
rrnB_T2_terminator	690 - 717	
AmpR_promoter	757 - 785	
ORF frame 2	827 - 1667	
Ampicillin	827 - 1687	
f1_origin	1746 - 2052	
pBR322_origin	2279 - 2898	
pGEX_3_primer	3295 - 3317	
pBRrevBam_primer	3456 - 3475	
AraC	4536 - 3658	
ORF frame 1	4587 - 3658	
araO2	4566 - 4581	
araO1	4723 - 4734	
CAP_BS	4765 - 4778	
pBAD_fwd_primer	4770 - 4789	
AraI1I2	4775 - 4813	
ARA_promoter	4810 - 4838	
T7_transl_en_RBS	4879 - 4	

Unique restriction sites	
NdeI	11
KpnI	137
SacII	212
HindIII	305
PstI	1372
FspI	1391
AseI	1439
ClaI	3564
AflII	3863
NruI	4270
AgeI	4636
BamHI	4801

Article: Intein-based biosynthetic incorporation of unlabeled protein tags into isotopically labeled proteins for NMR studies. Zuger S et al. (Nat Biotechnol. 2005 Jun ; 23(6):736-40. Pubmed)

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Price: $65.00

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Plasmid 15335

pSKDuet01
Plasmid 12172

Plasmid 15335: pSKBAD2

Gene/insert name:	DnaE C-intein
Alternative names:	Intein
Insert size (bp):	117
Species of gene(s):	Nostoc punctiforme
Fusion proteins or tags:	GB1
Terminal:	C terminal on backbone
Vector backbone:	pBAD (Search Vector Database)
Backbone manufacturer:	Invitrogen but modified
Type of vector:	Bacterial expression
Backbone size (bp):	4771
Cloning site 5':	NdeI
Site destroyed during cloning:	No
Cloning site 3':	KpnI
Site destroyed during cloning:	No
5' Sequencing primer:	pBAD-fw (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	Low Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	View sequence
Plasmid Provided In:	DH5a
Principal Investigator:	Hideo Iwai
Terms and Licenses:	MTA

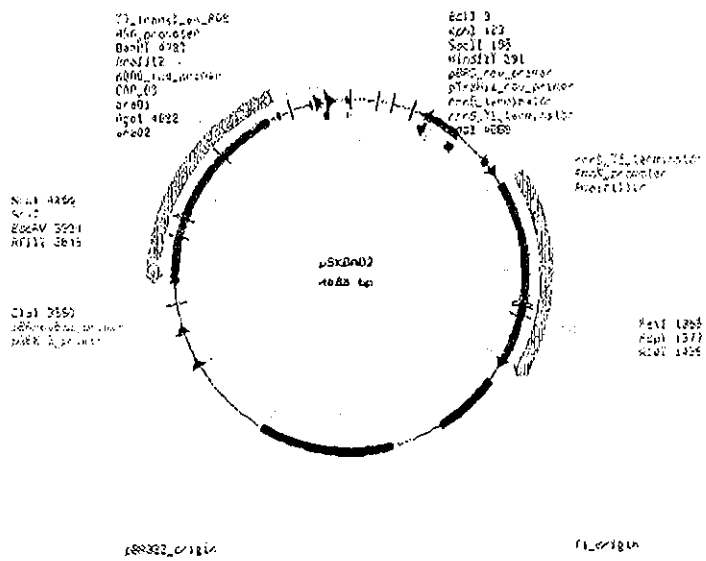
Plasmid Links
- Sequence
- Reviews (0)

Related Plasmids
- From this article
- Hideo Iwai Lab Plasmids

This is commonly requested with
- pSKDuet01

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

Click on map to enlarge



Selected features	
pBAD_rev_primer	346 - 329
pTrcHis_rev_primer	346 - 329
rrnB_terminator	379 - 535
rrnB_T1_terminator	501 - 544
rrnB_T2_terminator	676 - 703
AmpR_promoter	743 - 771
ORF frame 3	813 - 1673
Ampicillin	813 - 1673
f1_origin	1732 - 2038
pBR322_origin	2265 - 2884
pGEX_3_primer	3281 - 3303
pBRrevBam_primer	3442 - 3461
AraC	4522 - 3644
ORF frame 2	4573 - 3644
araO2	4562 - 4567
araO1	4709 - 4720
CAP_BS	4751 - 4764
pBAD_fwd_primer	4756 - 4775
AraI1I2	4761 - 4799
ARA_promoter	4796 - 4824
T7_transl_en_RBS	4865 - 4881

Unique restriction sites	
BclI	3
KpnI	123
SacII	198
HindIII	291
PstI	1356
FspI	1377
AseI	1425
ClaI	3550
AflII	3849
EcoRV	3934
NruI	4256
AgeI	4622
BamHI	4767
NdeI	4888

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
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 or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous 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 biohazards 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 Biohazard 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: GARY SHAW
SIGNATURE: GShaw
DEPARTMENT: BIOCHEMISTRY
ADDRESS: MSB 30b
PHONE NUMBER: x84021
EMERGENCY PHONE NUMBER(S): 519 858 2728
EMAIL: gshaw1@uwo.ca

Location of experimental work to be carried out: Building(s) MSB 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 its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR, Canadian Cancer Society
GRANT TITLE(S): Identification and mechanisms of novel S100 protein interactions
Structures and mechanisms of proteins involved in Parkinsons Disease
Structure and mechanism of class II E2 enzymes in ubiquitylation.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

Names of all personnel working under Principal Investigators supervision in this location:

Kathryn Barber
Don Spratt
Brian Dempsey
Mariena Silvestry
Atoosa Rezvanpour
Chantal Forrilstal
Ben Cook
Chee Wang Ng
Steven Beasley
Ventzi Hristova
Nicole Marlatt
Stephanie Serniwka

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓YES O NO
(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 ✓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)*	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 - BL21	O Yes ✓No	O Yes ✓No	O Yes ✓No	12 L	Invitrogen	✓1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*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? O YES ✓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 O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

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

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)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES ✓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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (preserved)		Not Applicable		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? ✓YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
JM109	pET pcDNA pGEX pDNR pJexpress	commercial		

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

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

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below ✓NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES ✓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? O 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 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

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

_____

_____

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

7.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs O YES ⊘NO
- Pound source cats O YES ⊘NO
- Cattle, sheep or goats O YES ⊘NO
- Non-human primates O YES, please specify species _____ ⊘NO
- Wild caught animals O YES, please specify species & colony # _____ ⊘NO
- Birds O YES ⊘NO
- Others (wild or domestic) O YES, please specify _____ ⊘NO

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____
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_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

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

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊘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 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:

_____
_____

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

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘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 NO, please forward the permit to he Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 ⊘NO

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ⓥ 1 O 2 O 3

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

ⓥ YES, permit # if on-campus Bio-UWO-2103

O NO, please certify

O NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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: 4 March 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

_____

_____

_____

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

_____

_____

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 9 April 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: April 9, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: Bio-UWO-0103 Expiry Date (3 years from Approval): April 8, 2013

Special Conditions of Approval:

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

Project Description for Biohazardous Agents Registry

Investigator: Gary Shaw

Projects Covered

A. Identification and Mechanisms of Novel S100 Protein Interactions (CIHR, 2009-2014)
B. Structures and Mechanisms of Proteins Involved in Parkinson's Disease (CIHR, 2009-2014)
C. Structure and Mechanism of Class II E2 Enzymes in Ubiquitylation

Brief Description of Biohazards Used

In all three projects a variety of plasmids are used as carriers for genes in non-infectious *E. coli* bacteria for the purpose of expressing specific proteins. Genes for a variety of S100 proteins including, but not limited to, S100B, S100A1, S100A11, S100A8, S100A9 and S100A10 are expressed in Project A. Genes for parkin, HOIL-1, S5a, UbcH7, UbcH8 are expressed in Project B. In Project C, genes for the proteins HIP2, Ubc1, cdc34 are expressed. In all cases, amino acid substitutions are, or might be made, for the purposes of structural and biophysical characterization of the resulting protein.

Identification and Mechanisms of Novel S100 Protein Interactions

Most S100 proteins are dimeric, *EF-hand* proteins that undergo calcium-induced conformational changes and interact with a variety of target proteins to trigger a biological response. However, the functional characterization of many S100 proteins is tempered by a lack of cellular data due to the transient nature of the calcium-bound S100 protein in vivo. In the past 2 years new and exciting *in vivo* data has unequivocally shown two major functions of the S100 proteins are (1) membrane vesiculation and repair and, (2) translocation of the G-coupled receptors to the plasma membrane. In (1) at least four proteins are required – S100A10, dysferlin, annexin A2 and the plasma membrane protein AHNAK – to bind to and close a lesion in a plasma membrane. In (2) S100A10 interacts with the third intracellular loop from the serotonin receptor 5HT1B and S100A10 knock-out mouse studies show a depression-like phenotype. S100B forms a complex with the same loop of dopamine receptors D2S and D2L and mediates signaling whose dysfunction leads to neurological disorders. *Importantly, all of these in vivo observations have occurred with calcium-free S100 proteins.* In addition, a new association between S100A11 and annexin A6 has also been identified that shows multiple binding sites indicating this protein-protein pair must have a unique role in membrane vesiculation events.

These observations have raised new questions about the biological roles and mechanisms of S100 proteins including: (1) can a constitutively active S100 protein be designed that would function *in vivo*, (2) how does S100A10 coordinate the assembly of dysferlin, AHNAK and annexin A2 to repair a membrane lesion, (3) what are the structures and mechanisms used by the serotonin and dopamine receptors for S100A10 and S100B recognition – can the S100B interaction be modulated by calcium binding and, (4) how does S100A11 recruit and position annexin A6 to control membrane fusion.

The **hypotheses** we wish to test are:
(1) Removal of S100A11 calcium sensitivity promotes a constitutively active conformation.
(2) S100A10 controls the assembly and disassembly of the dysferlin complex.
(3) S100A10 and S100B act as delivery proteins for serotonin and dopamine receptors to the plasma membrane that is mediated by annexin A2.
(4) S100A11 forms a novel structure with annexin A6 that can coordinate membrane fusion.

The **specific aims** and **research plan** are:
(1) Engineer hybrid apo-S100A11 proteins based on the "open" structure of S100A10. Assess the structures of these and use them to identify and confirm *in vivo* targets for S100A11,
(2) Identify the mechanism S100A10 uses to form a membrane repair complex that includes annexin A2, AHNAK and dysferlin. Determine the structure of this complex. Identify how the dysferlin V67D mutation disrupts complex formation.
(3) Determine the affinities and structures of S100A10 and S100B for the serotonin 5HT1B and dopamine D2L/D2S intracellular loops respectively. Identify the S100-receptor selectively and understand how annexin A2 modulates the S100-receptor interaction,
(4) Identify the binding regions of annexin A6 and mechanism used by S100A11 for interaction.

Our work will provide **significant advances** including:
(1) Development of a constitutively active S100A11 protein to be used for *in vivo* experiments,
(2) The first structural model of a protein complex that modulates membrane repair. A rationale for the dysferlin V67D mutation in limb girdle muscular dystrophy will be developed,
(3) The determinants of dopamine and serotonin receptor recognition for S100 proteins will be established allowing future drug design towards depression and neurological disorders,
(4) A new mechanism for membrane vesiculation used by S100A11 to recruit annexin proteins.

Structures and Mechanisms of Proteins Involved in Parkinson's Disease

Ubiquitylation is the central pathway for the trafficking of signaling proteins and removal of unfolded proteins from the cell. A defining step in this pathway is the passage of ubiquitin (Ub) or an ubiquitin-like (SUMO, ISG15) protein from an E2 enzyme (ie. UbcH8) to a substrate, complexed with an adaptor E3 ligase protein. Parkin is a key E3 ligase responsible for autosomal recessive juvenile parkinsonism (AR-JP), a common form of familial Parkinson's disease. This unique 465-residue protein comprises an N-terminal "ubiquitin-like" domain (UbLD) and a C-terminal RBR region consisting of two RING domains separated by an In-Between-RING domain (IBR). Missense mutations identified throughout parkin coincide with the disease. During the past grant period, we developed methods for parkin expression/purification, determined the 3D structure of parkin's IBR domain, and identified a new Zn-binding domain in parkin. We are now poised to use these novel findings to address the mechanisms of parkin-mediated protein degradation and trafficking including: (1) what mechanism does parkin use to distinguish association with the proteasome vs. substrates, (2) how does parkin recognize the E2 protein UbcH8 to ubiquitylate proteins, (3) how do UbcH8 and parkin facilitate ISG15 transfer in addition to Ub labeling, (4) what are the novel protein targets for parkin ubiquitylation and how are functions modified, and (5) what is the overall architecture of parkin and how do AR-JP mutations alter this? These questions will be addressed using biophysical, in vivo techniques and parkin carrying missense mutations to uncover the structures, interactions and mechanisms of this E3 ligase in neurodegeneration.

The **hypotheses** to be tested are:

(i) the UbLD forms distinctive, non-identical complexes with the proteasome vs. substrates,
(ii) UbcH8 binding and ubiquitylation activity requires the IBR-RING2 domain from parkin,
(iii) specific interactions occur between UbcH8, ISG15 and parkin to facilitate ISG15 transfer,
(iv) parkin selects and ubiquitylates cellular targets critical for dopamine neuron survival,
(v) a unique arrangement of parkin's UbLD and RING domains accounts for ubiquitylation.

The **specific aims** and **research plan** for this project are;

(i) determine 3D structures of parkin complexes with regions from the S5a proteasomal subunit and substrates ataxin-3 and Eps15,
(ii) identify the interactions and 3D structure of the parkin-UbcH8 complex,
(iii) explore the kinetics of Ub and ISG15 transfer by UbcH8 and parkin; determine structures of UbcH8-ISG15 and UbcH8-Ub,
(iv) identify biological targets for parkin by protein array and TAP tag methods; examine sub-cellular localization and functional outcomes,
(v) identify the spatial relationship and architecture of the UbLD and RBR regions of parkin.

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

(i) the first structures showing how parkin distinguishes proteasome vs substrate proteins,
(ii) the residues and surfaces on parkin allowing its interaction and function with UbcH8,
(iii) a structural rationale and mechanism for ISG-15 recognition and manipulation by parkin,
(iv) the interactome, subcellular localization and novel functions for parkin,
(v) the first 3D model of parkin allowing its mechanisms for ubiquitylation, and detrimental affects of missense mutations in AR-JP to be established.

10. DETAILED SCIENTIFIC ABSTRACT / RÉSUMÉ SCIENTIFIQUE DÉTAILLÉ

Responses must be limited to one page. Refer to the *2006 Grant Application Guide* for specific instructions regarding the format to be used for this section. / Les réponses doivent être limitées à une page. Consultez le *Guide de demande de subvention de 2006* pour obtenir les directives spécifiques ayant trait au format à utiliser pour cette section.

Shaw, Gary S.
Department of Biochemistry
The University of Western Ontario

Structure and Mechanism of Class II E2 Enzymes in Ubiquitylation

Keywords/Technical Terms: ubiquitin (Ub), conjugating enzymes, protein degradation, nuclear magnetic resonance (NMR) spectroscopy

The uncontrollable growth and proliferation of cancer cells is a result of defects in the cell cycle, the central process that insures proteins are phosphorylated and dephosphorylated in a timely and specific manner as required for transcription and translation events. This requires a delicate balance between the synthesis of cyclins, cyclin-dependent kinases (Cdk1, Cdk2) and kinase inhibitors (p21, p27) that perform the various signal transduction tasks of the cycle and the degradation of these same proteins to limit their function. It is now well-established that the ubiquitin-mediated proteolysis pathway (ubiquitylation) is the key process for optimizing the levels of most cell cycle proteins. For example, the G1-S transition of cell cycle is marked by Cdk2 activation as the cell prepares for DNA synthesis. This step is controlled, not by increased expression of Cdk2, but rather the degradation of the Cdk2 inhibitor p27 by an E3-ligase SCF complex that includes the E2-conjugating enzyme CDC34. In prostate, breast and colorectal cancers inappropriate reduction of p27 levels by over-active SCF ubiquitylation has been established based on mouse and clinical studies.

The E2 enzyme CDC34 is a central protein involved in the ubiquitylation pathway that accepts ubiquitin (Ub) from an E1 enzyme and forms an unstable Ub~E2 thiolester intermediate, either separately or as part of the SCF complex. Although many elegant biochemical studies and 3D structures of E1-E2 and E2-E3 complexes are available, the mechanism whereby these enzymes build polyubiquitin chains necessary to label a protein targeted for degradation is largely unknown. This is particularly true for the class II E2 conjugating enzymes such as CDC34, Hip2 and yeast Ubc1. These proteins are characterized by unique C-terminal extensions that are required for polyubiquitin chain formation and allow them to assemble these Ub polymers even in the absence of an E3 protein. We have recently determined the first structure of a class II conjugating protein (Ubc1) and have developed a novel method to create stable Ub-E2 intermediate protein complexes. We will now use this experience to focus on the E2 enzymes CDC34, Hip2 and Ubc1 and gain a detailed structural understanding of their structures and mechanisms of polyubiquitin chain assembly.

In particular we will address the following questions;

1) How does the Ub~E2 thiolester intermediate promote polyubiquitin chain assembly?
2) How do class II E2 enzymes such as Hip2, CDC34 and Ubc1 build polyubiquitin chains ?
3) How are K48- and K63-linked polyubiquitin chain recognized by Hip2 and Ubc1 ?

To answer these questions we will:

1) Determine the 3D structures of the Ub-E2 intermediates for CDC34, Hip2 and Ubc1 to identify how these unique class II E2 proteins direct ubiquitination.
2) Identify how CDC34, Hip2, and Ubc1 facilitate the transfer of a thioester-bound Ub to an acceptor Ub.
3) Determine the structure and provide a rationale for the involvement of the C-termini for Hip2 and Ubc1 for both the recruiting and assembly of K48- and K63-linked ubiquitin chains.

We anticipate these experiments will provide us with detailed structures and mechanisms showing how these proteins build polyubiquitin chains. Further we expect the differences observed between the structures and their interactions with assembled polyubiquitin chains will establish how a protein such as CDC34 efficiently labels p27 for proteasomal degradation. These details will be important in understanding how new therapies against cancer can be designed that target the E2 proteins.

Revision Date: 24-Mar-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 500149
Product name BL21 (DE3) One Shot

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	10-30

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available
Ingestion No information available

Specific effects

Carcinogenic effects No information available
Mutagenic effects No information available
Reproductive toxicity No information available

Sensitization No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects No information available.
Mobility No information available.
Biodegradation No information available.
Bioaccumulation No information available

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name Not classified as dangerous in the meaning of transport regulations
Hazard Class No information available
Subsidiary Class No information available
Packing group No information available
UN-No No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Glycerol	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

MATERIAL SAFETY DATA SHEET

NEW ENGLAND BioLabs Inc.
New England Biolabs
32 Tozer Road
Beverly MA 01915

EMERGENCY TELEPHONE NO. 1-800-632-5227
OTHER INFORMATION CALLS 1-978-927-5054
FAX: 1-978-921-1350
INTERNET e-mail: info@neb.com

Strain
#E4107S

SECTION 1 - PRODUCT

Product Name: *E. coli* K12 JM109

SECTION 2–COMPOSITION/ INFORMATION ON INGREDIENT

Strains supplied by NEB are all derivatives of *E. coli* K12, *E. coli* B or hybrids of these two strains. *E. coli* K12 and B are nonpathogenic isolates. K12 is the standard nonpathogenic host, exempt from the NIH Recombinant DNA Advisory Committee (RAC) guidelines (1).

E. coli B has also been shown to lack common pathogenicity-related sequences (2).

References:

1. Federal Register, (1986) Vol. V1: 88, 6952–16985.
2. Kuhnert, P., Hacker, I. Muldorfer, A. P. Burnens, J. Nicolet, and J. Frey (1997). Detection system for Escherichia coli-specific virulence genes.: absence of virulence determinants in B and C strains. Appl. Environ. Microbiol. 63(2): 703– 709.

Novagen®

Material Safety Data Sheet

Order Number **Customer Number**

1. Product and Company Identification

Supplier

Manufactured by EMD Biosciences, Inc.
441 Charmany Drive
Madison, WI 53719
(608)238-6110
(800)207-0144
FAX: (608)238-1388

P.O. Box 12087
La Jolla, CA 92039-2087
(858)450-5558
(800)854-3417
FAX: (858)453-3552

Catalog # 70777

In Case of Emergency Call Chemtrec®
(800)424-9300 (within U.S.A.)
(703)527-3887 (outside U.S.A.)

Product name pET Expression System 28

2. Composition and Information on Ingredients

Ingredient Name	CAS No.	Product No.	EU Symbol	R-Phrases
Induction Control E		RC0039	-	-
pET-28a(+) DNA		RC0041	-	-
BL21 Glycerol Stock		RC0131	-	-
BL21(DE3) Glycerol Stock		RC0262	-	-
BL21(DE3)pLysS Glycerol Stock		RC0263	-	-
pET-28b(+) DNA		RC0324	-	-
pET-28c(+) DNA		RC0325	-	-

Note: See section 8 for occupational exposure limits and section 11 for LC50/LD50 information.

3. Hazards Identification

Primary Hazards and Critical Effects	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	No specific hazard.
Physical/Chemical hazards	:		Not applicable.
Human Health Hazards	:		Not applicable.
Environmental Hazards	:		Not applicable.

4. First Aid Measures

Inhalation	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Ingestion	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Do NOT induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. If large quantities of this material are swallowed, call a physician immediately.
Skin Contact	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
Eye Contact	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.
Notes to Medical Doctor	:		Not available.

5. Fire-Fighting Measures

Extinguishing Media	:	Use foam or all purpose dry chemicals to extinguish.
Fire-Fighting Procedures	:	Fire fighters should wear positive pressure self-contained breathing apparatus (SCBA) and full turnout gear.
Fire/Explosion Hazards	:	Not applicable.
Hazardous Decomposition Products	:	Not available.

6. Accidental Release Measures

Personal Precautions	:	Immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable protective equipment (Section 8). Follow all fire fighting procedures (Section 5).
Environmental Precautions and Clean-up Methods	:	If emergency personnel are unavailable, contain spilled material. For small spills add absorbent (soil may be used in the absence of other suitable materials) scoop up material and place in a sealed, liquid-proof container for disposal. For large spills dike spilled material or otherwise contain material to ensure runoff does not reach a waterway. Place spilled material in an appropriate container for disposal. Minimize contact of spilled material with soils to prevent runoff to surface waterways.

Note: See section 1 for emergency contact information and section 13 for waste disposal.

7. Handling and Storage

Handling	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Wash thoroughly after handling.
Storage	:	Keep container tightly closed. Keep container in a cool, well-ventilated area.	
Packaging Materials	:	Use original container.	

8. Exposure Controls and Personal Protection

Occupational Exposure Limits

Ingredient Name	Occupational Exposure Limits
RC0039	Not available.
RC0041	Not available.
RC0131	Not available.
RC0262	Not available.
RC0263	Not available.
RC0324	Not available.
RC0325	Not available.

Engineering Controls	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	No special containment is required. Local exhaust ventilation should be provided.

Personal Protective Equipment

Respiratory System	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Use appropriate respiratory protection if there is the potential to exceed the exposure limit(s).
Skin and Body	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Work uniform or laboratory coat.
Hands	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Use chemical resistant, impervious gloves. Additional body garments should be used based upon the task being performed (e.g., sleevelets, apron, gauntlets, disposable suits). Appropriate techniques should be used to remove potentially contaminated clothing.
Eyes	:	RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	Safety glasses. Goggles, face shield, or other full-face protection if potential exists for direct exposure to aerosols or splashes.

9. Physical and Chemical Properties

Kit Components

39258: 1 x 0.2 ml Induction Control E (RC0039)
39386: 1 x 0.2 ml BL21 Glycerol Stock (RC0131)
39387: 1 x 0.2 ml BL21(DE3) Glycerol Stock (RC0262)
39388: 1 x 0.2 ml BL21(DE3)pLysS Glycerol Stock (RC0263)
39864: 1 x 10 ug pET-28a(+) DNA (RC0041)
39865: 1 x 10 ug pET-28b(+) DNA (RC0324)
39866: 1 x 10 ug pET-28c(+) DNA (RC0325)

Flash Point

Not available.

10. Stability and Reactivity

Stability	:	, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325	The product is stable.
Conditions and Materials to Avoid	:		Not available.
Hazardous Decomposition Products	:		Not available.

11. Toxicological Information

Toxicity Data

Ingredient Name	Test	Result	Route	Species
RC0039	Not available.	Not available.	Not available.	Not available.
RC0041	Not available.	Not available.	Not available.	Not available.
RC0131	Not available.	Not available.	Not available.	Not available.
RC0262	Not available.	Not available.	Not available.	Not available.
RC0263	Not available.	Not available.	Not available.	Not available.
RC0324	Not available.	Not available.	Not available.	Not available.
RC0325	Not available.	Not available.	Not available.	Not available.

Routes of Entry : Not available.

Acute Effects

Inhalation	:	Not available.
Ingestion	:	Not available.
Skin Contact	:	Not available.
Eye Contact	:	Not available.

Chronic Effects

Adverse Effects	:	Not available.
Target Organs	:	Not available.
Carcinogenic Effects	:	Not available.
Mutagenic Effects	:	Not available.
Developmental and Teratogenic Effects	:	Not available.
Reproductive Effects	:	Not available.

Other Information : RC0039, RC0041, RC0131, RC0262, RC0263, RC0324, RC0325 — Repeated or prolonged exposure is not known to aggravate medical condition.

12. Ecological Information

Ecotoxicity Data

Ingredient Name	Species	Period	Result
RC0039	Not available.	Not available.	Not available.
RC0041	Not available.	Not available.	Not available.
RC0131	Not available.	Not available.	Not available.
RC0262	Not available.	Not available.	Not available.
RC0263	Not available.	Not available.	Not available.
RC0324	Not available.	Not available.	Not available.
RC0325	Not available.	Not available.	Not available.

13. Disposal Consideration

Waste Handling and Disposal : Waste must be disposed of in accordance with federal, state and local environmental control regulations.

14. Transport Information

Air

IATA-DGR Class : Not controlled under IATA.

Packing Group

15. Regulatory Information

EU Regulations

Hazard Symbol(s) : -

Risk Phrases : This product is not classified according to the EU regulations.

Safety Phrases : Not applicable.

US Regulations

Haz-Com Standard : Not controlled under the HCS (United States).

EPA : Not available.

State : Not available.

Canadian Regulations

WHMIS : Not controlled under WHMIS (Canada).

CEPA : No products were found.

Provincial : No products were found.

16. Other Information

Validated by map on 10/17/2003.

Version : 1.0

Date of Printing : 10/20/2003.

Notice to Reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above named supplier nor any of its subsidiaries assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
*Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist. **This product is intended for research use only.***

Material Safety Data Sheet

Canada
English

Section 1. Chemical product and company identification

Product name pGEX-6P-1 Vector, 25 µg

Catalogue Number 28-9546-48

9 0 2 8 9 5 4 6 4 8

Material uses	Industrial applications: Analytical chemistry. Research.
Product type	Liquid.
Validation date	16 April 2009
Print date	16 April 2009
Supplier	GE Healthcare UK Ltd Amersham Place Little Chalfont Buckinghamshire HP7 9NA England +44 0870 606 1921

In case of emergency	US	ChemTrec (US)	1-800-424-9300
	Canada	ChemTrec (US)	1-703-527-3887

2. Hazards identification

Physical state	Liquid.
Odor	Odorless.
Emergency overview	No specific hazard. NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED. No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
Routes of entry	Dermal contact. Eye contact. Inhalation. Ingestion.

Potential acute health effects

Eyes	No known significant effects or critical hazards.
Skin	No known significant effects or critical hazards.
Inhalation	No known significant effects or critical hazards.
Ingestion	No known significant effects or critical hazards.

Potential chronic health effects

Chronic effects	No known significant effects or critical hazards.
Carcinogenicity	No known significant effects or critical hazards.
Mutagenicity	No known significant effects or critical hazards.
Teratogenicity	No known significant effects or critical hazards.
Developmental effects	No known significant effects or critical hazards.
Fertility effects	No known significant effects or critical hazards.
Target organs	Not available.
Inhalation	No specific data.
Ingestion	No specific data.
Skin	No specific data.
Eyes	No specific data.
Medical conditions aggravated by over-exposure	None known.

See toxicological information (section 11)

There are no ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Section 4. First aid measures

Eye contact	Check for and remove any contact lenses. Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical attention if symptoms occur.
Skin contact	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if symptoms occur.
Inhalation	Move exposed person to fresh air. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention if symptoms occur.
Ingestion	Wash out mouth with water. Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if symptoms occur.
Protection of first-aiders	No action shall be taken involving any personal risk or without suitable training.

Section 5. Fire fighting measures

Flammability of the product	In a fire or if heated, a pressure increase will occur and the container may burst.
Extinguishing media	
Suitable	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	None known.
Special exposure hazards	Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training.
Special protective equipment for fire-fighters	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up	Stop leak if without risk. Move containers from spill area. Prevent entry into sewers, water courses, basements or confined areas. Wash spillages into an effluent treatment plant or proceed as follows. Contain and collect spillage with non-combustible, absorbent material e.g. sand, earth, vermiculite or diatomaceous earth and place in container for disposal according to local regulations (see section 13). Dispose of via a licensed waste disposal contractor. Note: see section 1 for emergency contact information and section 13 for waste disposal.
Small spill	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

Section 7. Handling and storage

Handling	Put on appropriate personal protective equipment (see section 8). Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking.
Storage	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

Section 8. Exposure controls/personal protection

Consult local authorities for acceptable exposure limits.

Recommended monitoring procedures	If this product contains ingredients with exposure limits, personal, workplace atmosphere or biological monitoring may be required to determine the effectiveness of the ventilation or other control measures and/or the necessity to use respiratory protective equipment.
Engineering measures	No special ventilation requirements. Good general ventilation should be sufficient to control worker exposure to airborne contaminants. If this product contains ingredients with exposure limits, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure below any recommended or statutory limits.

potentially contaminated clothing. Wash contaminoted clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Personal protection

Respiratory	Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Hands	Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Eyes	Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists or dusts.
Skin	Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.
Environmental exposure controls	Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Section 9. Physical and chemical properties

Physical state	Liquid.
Color	Colorless.
Odor	Odorless.
pH	7.5 [Conc. (% w/w): 100%]
Volatility	0% (v/v)
VOC	0 (g/l).
Solubility	Easily soluble in the following materials: cold water and hot water.

Section 10. Stability and reactivity

Stability	The product is stable.
Materials to avoid	No specific data.
Hazardous polymerization	Under normal conditions of storage and use, hazardous polymerization will not occur.
Conditions of reactivity	Non-flammable in the presence of the following materials or conditions: open flames, sparks and static discharge, heat, shocks and mechanical impacts, oxidizing materials, reducing materials, combustible materials, organic materials, metals, acids, alkalis and moisture. Not considered to be a product presenting a risk of explosion.

Section 11. Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Not available.				

Conclusion/Summary: Not available.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
Not available.						

Synergistic products: Not available.

Section 12. Ecological information

Environmental effects	No known significant effects or critical hazards.
Octanol/water partition coefficient	Not available.
Bioconcentration factor	Not available.
Other adverse effects	No known significant effects or critical hazards.

Section 13. Disposal considerations

Waste disposal	The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in a safe way. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.
RCRA classification	Not available.

Disposal should be in accordance with applicable regional, national and local laws and regulations.

Section 14. Transport information

International transport regulations

Not classified.

Section 15. Regulatory information

WHMIS (Canada)	Not controlled under WHMIS (Canada).
Canadian lists	**CEPA Toxic substances:** None of the components are listed. **Canadian ARET:** None of the components are listed. **Canadian NPRI:** None of the components are listed. **Alberta Designated Substances:** None of the components are listed. **Ontario Designated Substances:** None of the components are listed. **Quebec Designated Substances:** None of the components are listed.
Canada inventory	All components are listed or exempted.

This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all the information required by the Controlled Products Regulations.

EU regulations

Hazard symbol or symbols	
Risk phrases	This product is not classified according to EU legislation.
Safety phrases	Not applicable.

International regulations

International lists	**Australia inventory (AICS):** All components are listed or exempted. **China inventory (IECSC):** All components are listed or exempted. **Japan inventory (ENCS):** All components are listed or exempted. **Japan inventory (ISHL):** Not determined. **Korea inventory (KECI):** All components are listed or exempted. **New Zealand Inventory of Chemicals (NZIoC):** All components are listed or exempted. **Philippines inventory (PICCS):** All components are listed or exempted.

Section 16. Other information

The customer is responsible for determining the PPE code for this material.

Indicates information that has changed from previously issued version.

History

Date of printing	16 April 2009	**Date of previous issue**	16 April 2009
Date of issue	16 April 2009	**Version**	1

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Clone: HsCD00004370

Clone ID:	HsCD00004370	Type:	cDNA
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
	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
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
	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

ost 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

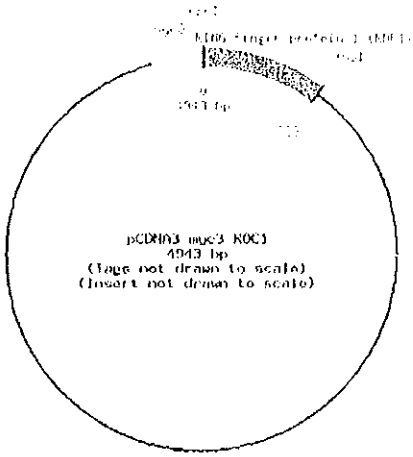
AF142059
NCBI: RBX1
RBX1 antibodies

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.

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Protein Structure Initiative - Material Repository (PSI-MR)

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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

• Plasmid Submission •

Protein expression.

Overview
Expression Guarantee
Synthetic Gene Expression
Design Parameters
Optimized Genes
Host Systems
Expression Vectors

Place Order or Request Quote

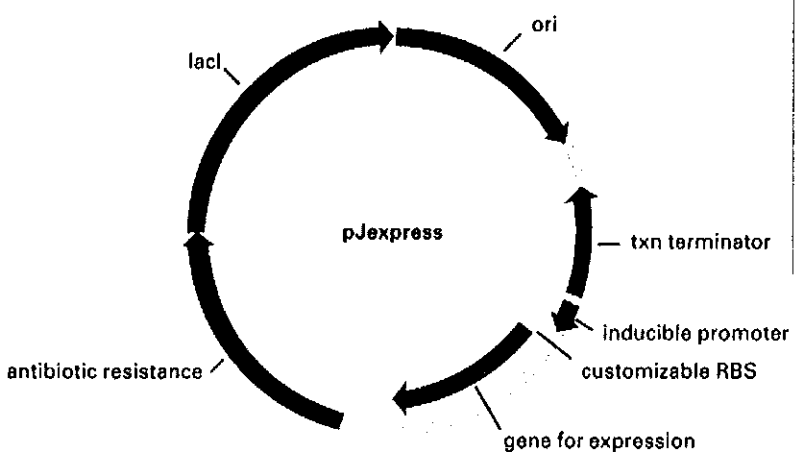
Protein Expression Vectors

Vectors for bacterial expression

DNA2.0 synthetic genes are available in vectors that are immediately usable for bacterial protein expression. Cloning into these vectors is performed at no additional cost and the constructs are provided without IP restrictions. DNA2.0 can even guarantee that your gene will express.

pJexpress vectors transcribe from promoters that are repressed by flanking copies of the lac operator. The vectors also carry a copy of the lacI gene, which expresses the repressor protein that binds to the lac operator.

pJexpress Vector Map



pJexpress Features

- Higher level of protein expression than pET vectors when using the same insert (see gel below).
- Choose between ampicillin, kanamycin, chloramphenicol and zeocin resistances
- 'IP-free'. The T5 promoter sequence was described in 1985 (Gentz and Bujard (1985) J. Bacteriol **164**;70) and we have not patented our modifications. pJexpress vectors with T5 promoter can be induced by IPTG in any *E. coli* host.
- Silencing of the promoter prior to IPTG induction is achieved using symmetrical lac operators (Sadler *et al* (1983) Proc Natl Acad Sci USA **80**;6785) spaced around the promoter to maximize cooperativity (Oehler *et al* (1994) EMBO J 13;3348). This operator pair ensures significantly tighter repression than regular lac operators. Overlapping T5 promoter/lac operator has been described (Lanzer and Bujard 1988 Proc Natl Acad Sci USA 85:8973).
- Customize your own ribosome binding site (RBS), have us design one for you or use the default RBS tested in gel below (AGGAGGTAAAACAT).

pJexpress vectors are currently available with the following options

Vector	Features
pJexpress 401	T5 promoter[A], Kan[r], pUC origin[C]

pJexpress 402	T5 promoter[A], Zeo^r, pUC origin[C]
pJexpress 404	T5 promoter[A], Amp^r, pUC origin[C]
pJexpress 406	T5 promoter[A], Kan^r, Amp^r, Clm^r, pUC origin[C]
pJexpress 411	T7 promoter[B], Kan^r, pUC origin[C]
pJexpress 412	T7 promoter[B], Zeo^r, pUC origin[C]
pJexpress 414	T7 promoter[B], Amp^r, pUC origin[C]
pJexpress 416	T7 promoter[B], Kan^r, Amp^r, Clm^r, pUC origin[C]

[A] We have modified the T5 promoter to give even higher protein expression levels; it is repressible, and works in any *E. coli* strain.

[B] The T7 promoter results in high protein expression levels and is repressible. The T7 promoter only works in T7 pol expressing *E. coli* strains (eg. BL21(DE3) or T7 Express). Note that strains in which basal expression is reduced, such as those carrying lysS or lysY, frequently express to lower levels after induction when compared with strains that carry only the T7 polymerase gene. Use of host cells that may contain the cloned copy of the T7 gene 1, the gene for T7 RNA polymerase with any other vector(s) containing a T7 promoter to direct the production of RNA or protein requires a license from Brookhaven National Laboratory. Information about research-use or commercial-use license agreements may be obtained from the Office of Intellectual Property and Sponsored Research, Brookhaven National Laboratory, Building 475D, P.O. Box 5000, Upton, New York, 11973-5000; telephone: 631-344-7134; fax: 631-344-3729.

[C] pUC origin of replication results in high copy number plasmid.

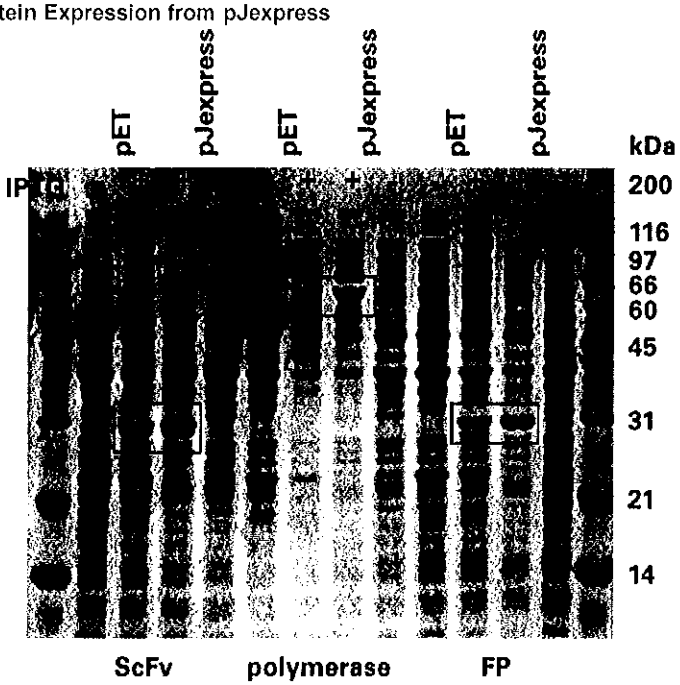
For PCR or sequencing primers flanking the insert we use the following oligonucleotides:
pTF 5'-CTCGAAAATAATAAAGGGAAAATCAG-3'
pTR 5'-TGGTAGTGTGGGGACTC-3'

Please note: The pJ vectors do not contain restriction sites for excision of your gene. These must be included in your gene design if you wish to remove your gene with restriction enzymes.

pJexpress Tags

pJexpress vectors do not come with built in tags for affinity purification or solubility enhancement. Any tag (plus any protease cleavage site for tag removal) can be added very cost effectively at either the N- or C-terminus during synthesis.

Protein Expression from pJexpress



Genes encoding a single chain antibody (ScFv ~30 kDa), a DNA polymerase (polymerase ~65 kDa), or a fluorescent protein (FP ~30 kDa) were designed using DNA2.0's algorithms for codon optimization to express in *E. coli*. The genes were cloned into pJexpress401 or pET28 then transformed into an *E. coli* host for expression. Genes in pJexpress401 were transformed into standard cloning strain DH10B; genes in pET28 were transformed into a

host strain expressing T7 RNA polymerase, in this case BL21(DE3)-pLysS. One colony of each construct was picked and grown in 3 ml LB containing the appropriate antibiotic (in this case 25 µg/ml kanamycin). Cells were grown at 37°C with shaking until they had reached an A_{600} between 0.6 and 1.0. An aliquot of culture (1 ml) was then taken into 3 ml of LB containing antibiotic plus 1 mM IPTG, and grown for a further 4 hours. At this time, samples (50 µl) were taken from uninduced (-) and IPTG induced (+) cultures, SDS sample buffer was added, and the samples were heated at 99°C for 10 minutes and the equivalent of 2 µl of culture was run on a 10% polyacrylamide gel.