The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards 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.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Gary Shaw**
DEPARTMENT:	**Biochemistry**
ADDRESS:	**Medical Sciences Building M306**
PHONE NUMBER:	**519-661-4021**
EMERGENCY PHONE NUMBER(S):	**519-859-2728**
EMAIL:	**gshaw1@uwo.ca**

Location of experimental work to be carried out :

Building :	**Medical Sciences**	Room(s):	**M312**
Building :		Room(s):	
Building :		Room(s):	

***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 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR**

GRANT TITLE(S): **1) Identification and Mechanisms of Novel S100 Protein Interatctions**
2) Structures and Mechanisms of Proteins Involved in Parkinson's Disease

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Kathy Barber	**kbarber@uwo.ca**	**13/09/06**
Don Spratt	**dspratt@uwo.ca**	**19/09/11**
Brian Dempsey	**bdempse2@uwo.ca**	**19/09/11**
Liliana Kisiel	**lsantam@uwo.ca**	**01/05/06**
Chee Ng	**cng92@uwo.ca**	**13/05/09**

Julia Noh	ynoh@uwo.ca	22/09/11
Jake Aguirre	jaguirre@uwo.ca	10/05/11
Tara Condos	tcondos@uwo.ca	10/05/11
Gary Shaw	gshaw1@uwo.ca	01/05/06

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

In both 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 the first project. In the second project genes for a variety of proteins including, but not limited to, parkin, HOIL-1, S5a, UbcH7, UbcH8, HIP2, Ubc1, Ub and 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. Frozen cell stocks are stored at -80C and after use are treated with bleach or autoclaved in Biohazard bags.

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

Calcium-binding Proteins

Projects focus on the three-dimensional structures of calcium-binding proteins and their roles in health and disease. We use physical biochemical techniques such as high resolution NMR spectroscopy, analytical centrifugation, fluorescence and circular dichroism to probe structure/function relationships.□□We are particularly interested in a group of proteins called the S100 proteins. These are dimeric signaling molecules belonging to the EF-hand calcium binding protein family. The proteins undergo a calcium-induced conformational change and interact with specific target proteins paralleling the mechanisms of the calcium sensor proteins troponin-C, calmodulin and recoverin. Recently we uncovered the conformational change in the protein S100B using multi-dimensional NMR spectroscopy. Currently we are probing the details of this structural change for S100B and other signalling proteins

Protein Interactions in Degradation and Parkinson's Disease

The process of ubiquitylation is one of the most important regulatory pathways in all cells. It involves the transfer of ubiquitin (Ub) between a series of proteins until it labels a target protein as a polyubiquitin chain. During the course of ubiquitylation, Ub first forms a high-energy thiolester intermediate with an active site cysteine of the activating enzyme E1 in an ATP-dependent step. The ubiquitin molecule is then transferred to the catalytic cysteine of an E2 conjugating enzyme (Ubc1, Hip2, UbcH7, UbcH8), forming a second thiolester. Labeling of a substrate protein occurs through transfer of the Ub directly from the E2 as in the case of RING E3 enzymes (cCbl, parkin) or through an intermediary transfer to a HECT domain E3. This mechanism is further complicated by a plethora of ubiquitin-like proteins (e.g. SUMO, NEDD8) and ubiquitin-binding domains (e.g. UBA, CUE, UIM) that have accessory functions such as targeting an ubiquitin complex to the proteasome.

Our lab is concentrating on the structures and mechanisms of Ub-E2-E3 complexes in order to define the molecular basis for ubiquitin chain elongation. We have determined the three-dimensional structure of Ubc1 (24 kDa), the first structure for a class II ubiquitin conjugating protein. We are now using our experience with this system to determine how the E2 enzymes Hip2, UbcH7, UbcH8 and Ubc1 interact with ubiquitin, and the E3 ligase proteins cCbl and parkin, enroute to polyubiquitin chain formation.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ NO
(non-pathogenic and pathogenic biological agents 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? ☐ 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:

Full Scientific Name of Biological Agent(s)* (Be specific)	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
BL21(DE3)	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**12**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
DH5-alpha	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.05**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
JM109	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.05**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
TOPP2	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**12**	**U. of Montreal**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
XL1-BLUE	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.05**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (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	☐ Yes ☐ No		Not applicable
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ 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 ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

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

3.2 Indicate 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☒ YES ☐ NO If **NO**, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
JM109 DH5-alpha XL1-blue	**pET pGex pETDuet pJExpress p11**	**commercial**	**S100 UbcH Ub cdc34 parkin AHNAK Annexin HIP2 HOIL S5a Rbx1 SMARC**	**no**	**no**	**none**

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☒ NO

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

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

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

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☐ YES ☒ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☐ YES, specify:

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

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

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

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ 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 insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree…)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

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

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

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 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 biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Gary Shaw ***Date:*** 22 Nov 2012

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ☐ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
- ☐ YES, location and date of most recent biosafety inspection:
- ☐ NO, please certify
- ☒ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-003**

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO

If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Treat immediately and report incident.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Gary Shaw ***Date:*** 22 Nov 2012

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Canadian Food Inspection Agency | Agence canadienne d'inspection des aliments

Office of Biohazard Containment and Safety
Science Branch, CFIA
59 Camelot Drive, Ottawa, Ontario K1A 0Y9
Tel: (613) 221-7068 Fax: (613) 228-6129
Email: ImportZoopath@inspection.gc.ca

Bureau du confinement des biorisques et sécurité
Direction générale des sciences, ACIA
59 promenade Camelot, Ottawa, Ontario K1A 0Y9
Tél: (613) 221-7068 Téléc: (613) 228-6129
Courriel: ImportZoopath@inspection.gc.ca

October 20th, 2009

Ms. Shamila Survery / Mr. Michael Decosimo
Cedarlane Laboratories Ltd
4410 Paletta Court
Burlington, Ontario L7L 5R2

By Facsimile: (289) 288-0020

SUBJECT: **Importation of *Escherichia coli* strains**

Dear Ms. Survery / Mr. Decosimo:

Our office received your query about the importation of *Escherichia coli* from the American Type Culture Collection (ATCC) located in Manassas, Virginia, United States. The following *Escherichia coli* strains are consider to be level 1 animal pathogens:

- 5K
- 58
- 58-161
- 679
- 1532
- AB284
- AB311
- AB1157
- AB1206
- AG1
- B
- BB4
- BD792
- BL21
- BL21 (DE3)
- BM25.8
- C
- C-1a
- C-3000
- C25
- C41 (DE3)
- C43 (DE3)
- C600
- Cavalli Hfr
- CIE85
- DH1
- DH10 GOLD
- DH10B
- DH5
- DH5-alpha
- DP50
- DY145
- DY380
- E11
- EJ183
- EL250
- EMG2
- EPI 300
- EZ10
- FDA Seattle 1946
- Fusion-Blue
- H1443
- HF4714
- HB101
- HS(PFAMP)R
- Hfr3000
- Hfr3000 X74
- HMS174
- J52
- J53
- JC3272
- JC7661
- JC9387
- JF1504
- JF1508
- JF1509
- JJ055
- JM83
- JM101
- JM109
- K12
- KC8
- KA802
- KAM32
- KAM33
- KAM43
- LE450
- LE451
- LE452
- MB408
- MBX1928
- MC1061
- MC4100 (MuLac)
- MG1655
- MM294
- MS101
- NC-7
- Nissle 1917
- One Shot STBL3
- OP50
- P678
- PA309
- PK-5
- PMC103
- PR13
- Rri
- RV308
- S17-1λ -PIR
- SCS1
- SMR10
- SOLR
- SuperchargeEZ10
- SURE
- TOP10
- TG1
- U5/41
- W208
- W945
- W1485
- W3104
- W3110
- WA704
- WP2
- X1854
- X2160T
- X2541
- X2547T
- XL1-BLUE
- XL1-BLUE-MRF
- XLOLR
- Y10
- Y1090 (1090)
- YN2980
- W3110
- WG1
- WG439
- WG443
- WG445

The Office of Biohazard Containment and Safety (BCS) of the Canadian Food Inspection Agency (CFIA) only issues import permits for microorganisms that are pathogenic to animals, or parts of microorganisms that are pathogenic to animals. As the products listed above are not considered pathogenic to animals, the Office of BCS does not have any regulatory requirements for their importation.

Please note that other legislation may apply. You may wish to contact the Public Health Agency of Canada's (PHAC) Office of Laboratory Security at (613) 957-1779.

Note: Microorganisms pathogenic to animals and veterinary biologics require an import permit from the CFIA.

Sincerely,

Cinthia Labrie

Cinthia Labrie
Head, Animal Pathogen Importation Program
Office of Biohazard Containment & Safety

Canada

Plasmid Files

pETDuet-1

Bacterial vector for the co-expression of two genes.

To see this sequence with restriction sites, features, and translations, please download SnapGene or the free SnapGene Viewer.

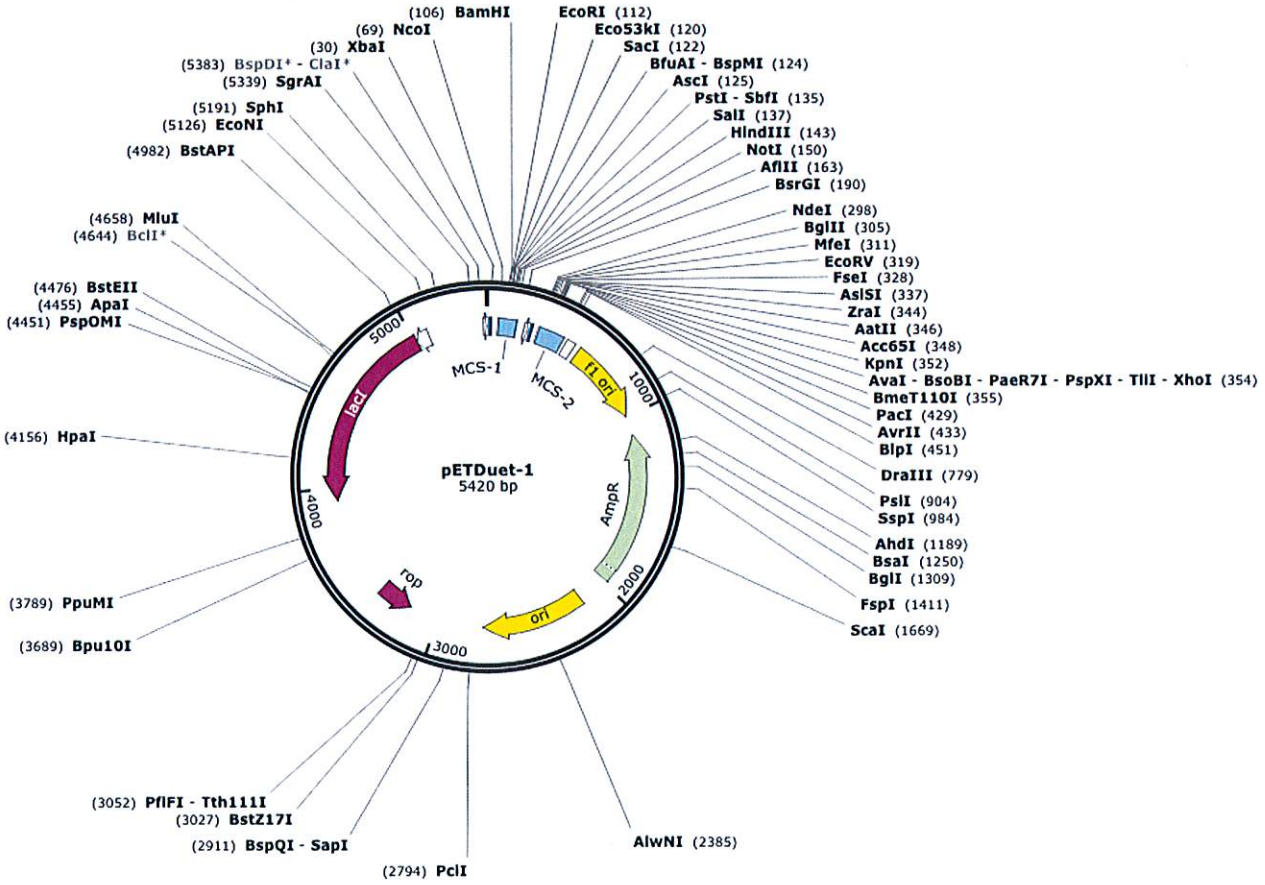
pETDuet-1.dna (Sequence and Map File | 40 KB)
Sequence Author: Novagen (EMD Millipore)

SnapGene Viewer 1.3.3 for Mac OS X (43 MB)

SnapGene Viewer 1.3.3 for Windows (13 MB)

Your time is valuable!
Basic Cloning Vectors
Fluorescent Protein Genes & Plasmids
Mammalian Expression Vectors
Viral Expression & Packaging Vectors
Yeast Plasmids
pET Vectors (Novagen)
pETDuet-1
pGEX Vectors (GE Healthcare)
Qiagen Vectors

Created with SnapGene™



Individual Sequences & Maps

pET-3a pET-21a(+) pET-28b(+) pET-32 Xa/LIC (linearized) pET-44c(+)

pET-3b	pET-21b(+)	pET-28c(+)	pET-33b(+)	pET-45b(+)
pET-3c	pET-21c(+)	pET-29a(+)	pET-39b(+)	pET-46 Ek/LIC
pET-3d	pET-21d(+)	pET-29b(+)	pET-40b(+)	pET-46 Ek/LIC (linearized)
pET-9a	pET-22b(+)	pET-29c(+)	pET-41a(+)	pET-47b(+)
pET-9b	pET-23(+)	pET-30a(+)	pET-41b(+)	pET-48b(+)
pET-9c	pET-23a(+)	pET-30b(+)	pET-41c(+)	pET-49b(+)
pET-9d	pET-23b(+)	pET-30c(+)	pET-41 Ek/LIC	pET-50b(+)
pET-11a	pET-23c(+)	pET-30 Ek/LIC	pET-41 Ek/LIC (linearized)	pET-51b(+)
pET-11b	pET-23d(+)	pET-30 Ek/LIC (linearized)	pET-42a(+)	pET-51b(+) Ek/LIC
pET-11c	pET-24(+)	pET-30 Xa/LIC	pET-42b(+)	pET-51b(+) Ek/LIC (linearized)
pET-11d	pET-24a(+)	pET-30 Xa/LIC (linearized)	pET-42c(+)	pET-52b(+)
pET-14b	pET-24b(+)	pET-31b(+)	pET-43.1a(+)	pET-52b(+) 3C/LIC
pET-15b	pET-24c(+)	pET-32a(+)	pET-43.1b(+)	pET-52b(+) 3C/LIC (linearized)
pET-16b	pET-24d(+)	pET-32b(+)	pET-43.1c(+)	pETDuet-1
pET-17b	pET-25b(+)	pET-32c(+)	pET-43.1 Ek/LIC	pLacI
pET-19b	pET-26b(+)	pET-32 Ek/LIC	pET-43.1 Ek/LIC (linearized)	pLysE
pET-20b(+)	pET-27b(+)	pET-32 Ek/LIC (linearized)	pET-44a(+)	pLysS
pET-21(+)	pET-28a(+)	pET-32 Xa/LIC	pET-44b(+)	

pET-28a-c(+) Vectors

TB074 12/98

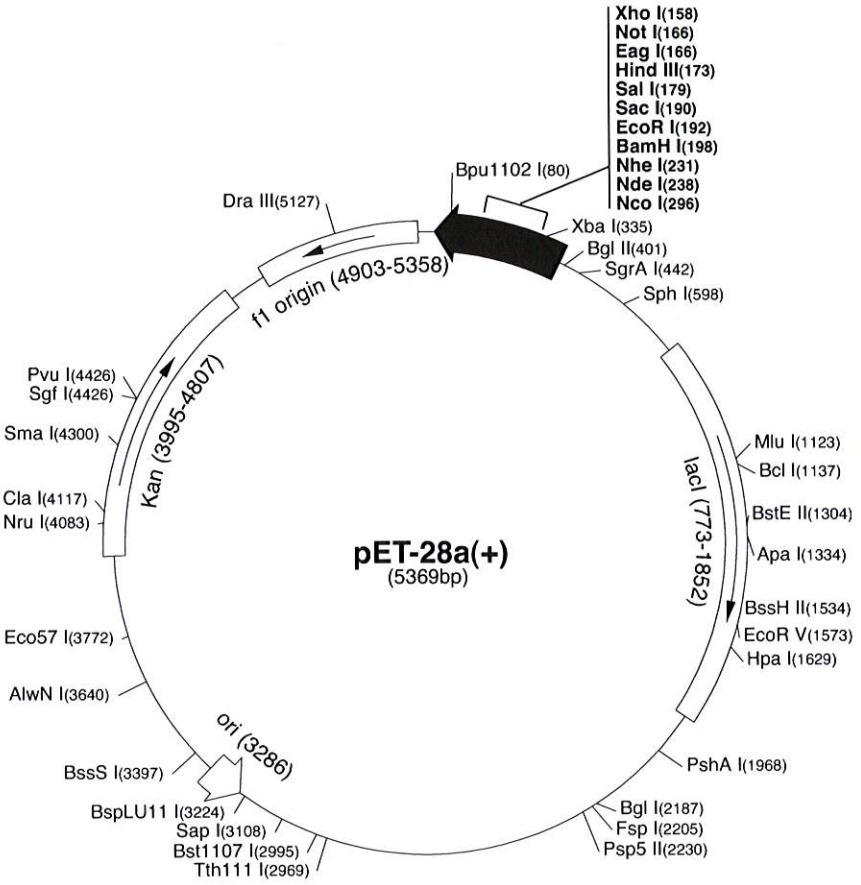
	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

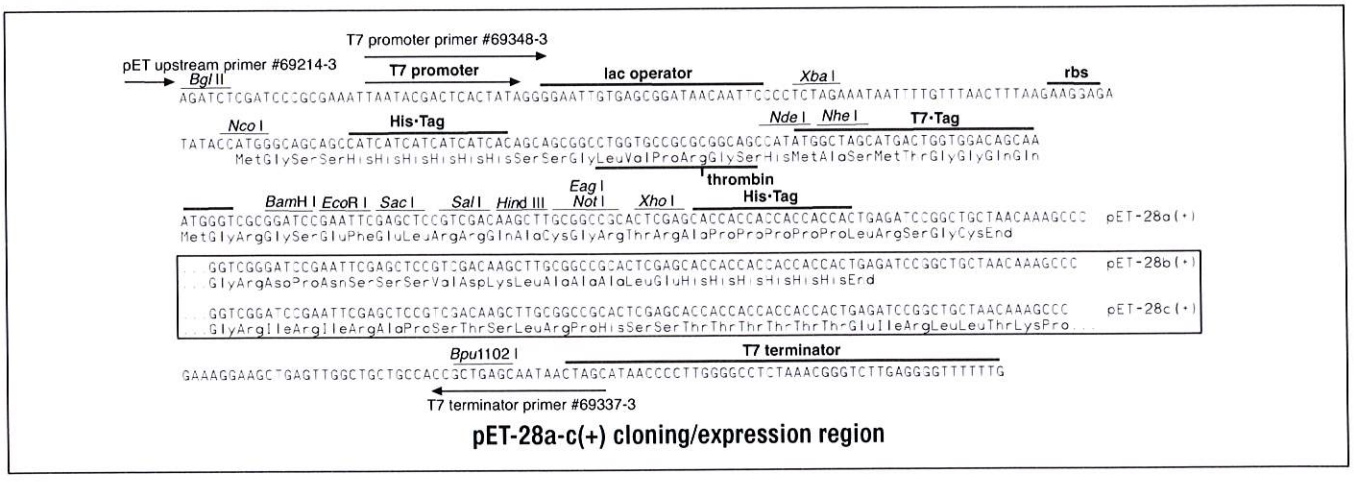
The pET-28a-c(+) vectors carry an N-terminal His•Tag®/thrombin/T7•Tag® configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single-stranded DNA that corresponds to the coding strand. Therefore, single-stranded sequencing should be performed using the T7 terminator primer (Cat. No. 69337-3).

pET-28a(+) sequence landmarks

T7 promoter	370-386
T7 transcription start	369
His•Tag coding sequence	270-287
T7•Tag coding sequence	207-239
Multiple cloning sites	
(*Bam*H I - *Xho* I)	158-203
His•Tag coding sequence	140-157
T7 terminator	26-72
lacI coding sequence	773-1852
pBR322 origin	3286
Kan coding sequence	3995-4807
f1 origin	4903-5358

The maps for pET-28b(+) and pET-28c(+) are the same as pET-28a(+) (shown) with the following exceptions: pET-28b(+) is a 5368bp plasmid; subtract 1bp from each site beyond *Bam*H I at 198. pET-28c(+) is a 5367bp plasmid; subtract 2bp from each site beyond *Bam*H I at 198.





pET-28a-c(+) cloning/expression region

pET-28a(+) Restriction Sites

TB074 12/98

Enzyme	# Sites	Locations				
AccI	2	180	2994			
AceIII	7	890	1618	1949	2733	2874
		3176	4967			
AciI	77					
AflIII	2	1123	3224			
AluI	22					
AlwI	13					
Alw21I	7	159	190	623	1107	2218
		3042	3542			
Alw44I	3	1103	3038	3538		
AlwNI	1	3640				
ApaI	1	1334				
ApaBI	1	807				
ApoI	6	192	1398	4039	4223	4929
		4940				
AvaI	2	158	4298			
AvaII	5	1675	2051	2139	2230	2509
BamHI	1	198				
BanI	9	253	445	466	580	1043
		1762	1892	2018	5164	
BanII	6	190	507	521	1334	4081
		5202				
BbsI	4	1269	1608	1982	2342	
BbvI	27					
BccI	14					
Bce83I	6	21	1937	2107	3315	3613
		3854				
BcefI	6	642	983	1610	3726	4745
		5153				
BcgI	9	160	194	228	1415	1449
		1949	1983	2801	2835	
BclI	1	1137				
BfaI	7	70	232	336	2238	3719
		4026	5278			
BglI	1	2187				
BglII	1	401				
BmgI	1	1332				
BpmI	4	961	1450	2084	2751	
Bpu10I	2	2330	4443			
Bpu1102I	1	80				
BsaAI	2	2976	5127			
BsaBI	3	400	406	2421		
BsaHI	5	446	467	581	1080	1763
BsaJI	10	57	296	560	566	1758
		2196	3384	4297	4298	4699
BsaWI	7	2	1442	1945	2413	3430
		3577	4561			
BsaXI	2	1782	5075			
BsbI	2	2940	5034			
BscGI	11					
BsgI	3	974	1174	2384		
BsiI	1	3397				
BsiEI	5	169	1908	3140	3564	4426
BsII	23					
BsmI	2	4310	4387			
BsmAI	6	820	1225	1351	1738	2865
		4442				
BsmBI	3	1738	2865	4442		
BsmFI	4	584	2125	2495	5342	
BsoFI	48					
Bsp24I	12					
Bsp1286I	12					
BspEI	2	2	2413			
BspGI	1	2750				
BspLU11I	1	3224				
BsrI	22					
BsrBI	4	356	3157	4825	5271	
BsrDI	2	1170	1536			
BsrFI	7	433	442	809	2021	2181
		4380	5228			
BssHII	1	1534				
Bst1107I	1	2995				

Enzyme	# Sites	Locations				
BstEII	1	1304				
BstXI	3	925	1054	1177		
BstYI	9	132	198	401	687	1899
		2416	3865	3876	4675	
Cac8I	40					
CjeI	26					
CjePI	30					
ClaI	1	4117				
CviJI	86					
CviRI	22					
DdeI	11					
DpnI	21					
DraIII	1	5127				
DrdI	3	2917	3332	5082		
DrdII	2	846	5132			
DsaI	3	296	560	2196		
EaeI	4	166	431	563	1797	
EagI	1	166				
EarI	3	741	3108	4239		
EciI	3	900	3298	3444		
Eco47III	3	528	2029	2478		
Eco57I	1	3772				
EcoNI	2	658	4338			
EcoO109I	3	53	556	2230		
EcoRI	1	192				
EcoRII	10	256	846	1161	1701	1758
		3250	3371	3384	4314	4671
EcoRV	1	1573				
FauI	17					
FokI	9	1169	1178	2443	2505	2583
		2769	2910	4064	4670	
FspI	1	2205				
GdiII	4	166	431	563	1797	
HaeI	6	851	2172	3239	3250	3702
		4513				
HaeII	14					
HaeIII	24					
HgaI	11					
HgiEII	2	721	3810			
HhaI	47					
Hin4I	3	1022	4112	4654		
HincII	2	181	1629			
HindIII	1	173				
HinfI	18					
HpaI	1	1629				
HphI	16					
MaeII	14					
MaeIII	16					
MboII	12					
MluI	1	1123				
MmeI	7	3439	3623	4068	4262	4624
		4633	5104			
MnlI	25					
MseI	25					
MslI	6	1175	1463	1493	2211	2406
		2797				
MspI	29					
MspA1I	9	84	264	1153	1723	1816
		2815	2934	3566	3811	
MwoI	39					
NarI	4	446	467	581	1763	
NciI	12					
NcoI	1	296				
NdeI	1	238				
NgoAIV	4	433	2021	2181	5228	
NheI	1	231				
NlaIII	26					
NlaIV	22					
NotI	1	166				
NruI	1	4083				
NsiI	2	4276	4542			
NspI	4	598	2569	2861	3228	

Enzyme	# Sites	Locations				
Pfl1108I	1	2010				
PflMI	2	705	4689			
PleI	9	384	672	759	1555	3118
		3603	4658	5062	5070	
PshAI	1	1968				
Psp5II	1	2230				
Psp1406I	4	785	2153	2549	4912	
PvuI	1	4426				
PvuII	3	1723	1816	2815		
RcaI	3	521	3944	4819		
RsaI	3	1270	3030	4261		
SacI	1	190				
SalI	1	179				
SapI	1	3108				
Sau96I	14					
Sau3AI	21					
ScrFI	22					
SfaNI	23					
SfcI	4	369	3489	3680	5346	
SgfI	1	4426				
SgrAI	1	442				
SmaI	1	4300				
SphI	1	598				
SspI	2	4351	4919			
StyI	2	57	296			
TaqI	15					
TaqII	6	1031	1249	1922	3126	4680
		5031				
TfiI	9	1802	2104	2274	2778	3199
		4337	4393	4565	4656	
ThaI	38					
TseI	27					
Tsp45I	7	1304	2132	2663	2876	2971
		4573	5300			
Tsp509I	20					
Tth111I	1	2969				
Tth111II	8	962	1655	2685	3814	3821
		3853	4262	4389		
UbaJI	21					
VspI	5	384	1808	1867	4625	4814
XbaI	1	335				
XcmI	3	979	1495	1513		
XhoI	1	158				
XmnI	2	2782	4815			

Enzymes that do not cut pET28a(+):

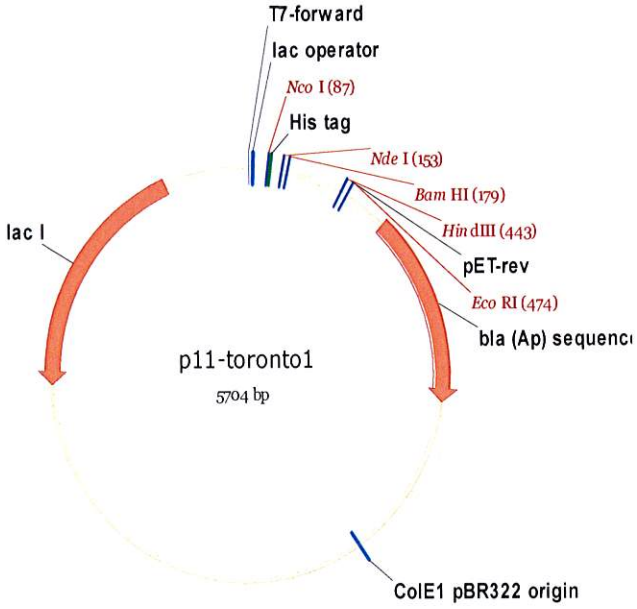
AatII	AflII	AgeI	AscI	AvrII
BaeI	BsaI	BseRI	BspMI	BsrGI
Bsu36I	DraI	Eam1105I	FseI	KpnI
MscI	MunI	NspV	PacI	PmeI
PmlI	PstI	RleAI	RsrII	SacII
ScaI	SexAI	SfiI	SnaBI	SpeI
SrfI	Sse8387I	StuI	SunI	SwaI

Vector information sheet.

Vector Name	**p11**
Source	Sujata Sharma, Toronto SGC
Sequence accession/link	(SGC)

Description	pET expression vector with His_6 tag in 22-aa N-terminal fusion peptide, with TEV protease cleavage site.

Antibiotic resistance	amp
Promoter	T7 - lacO
Cloning	In-frame NdeI – BamHI.
Initiation codon	In vector
N-terminal fusion – seq.	MGSSHHHHHHSSGRENLYFQ*GH (* - TEV cleavage site)
N-terminal fusion – MW	2693.07 Da (including met). TEV cleavage removes 2367.65 Da.
Termination codons	In vector, after BamHI (in frame: GGA TCC TAA; adds gly-ser to the C-terminus) (Termination codon may be supplied in the insert)
C-terminal fusion – seq.	GS
C-terminal fusion – MW	144.14 Da
Protease cleavage	TEV
Additional features	
Preferred host	DE3 hosts: BL21, Rosetta, etc. MUST express T7 RNA polymerase.
5' sequencing primer	T7 promoter: TAATACGACTCACTATAGGG
3' sequencing primer	PET-rev. ATGTTTGACAGCTTATCATCGA **NOTE: standard T7 terminator primer does not work!!!**



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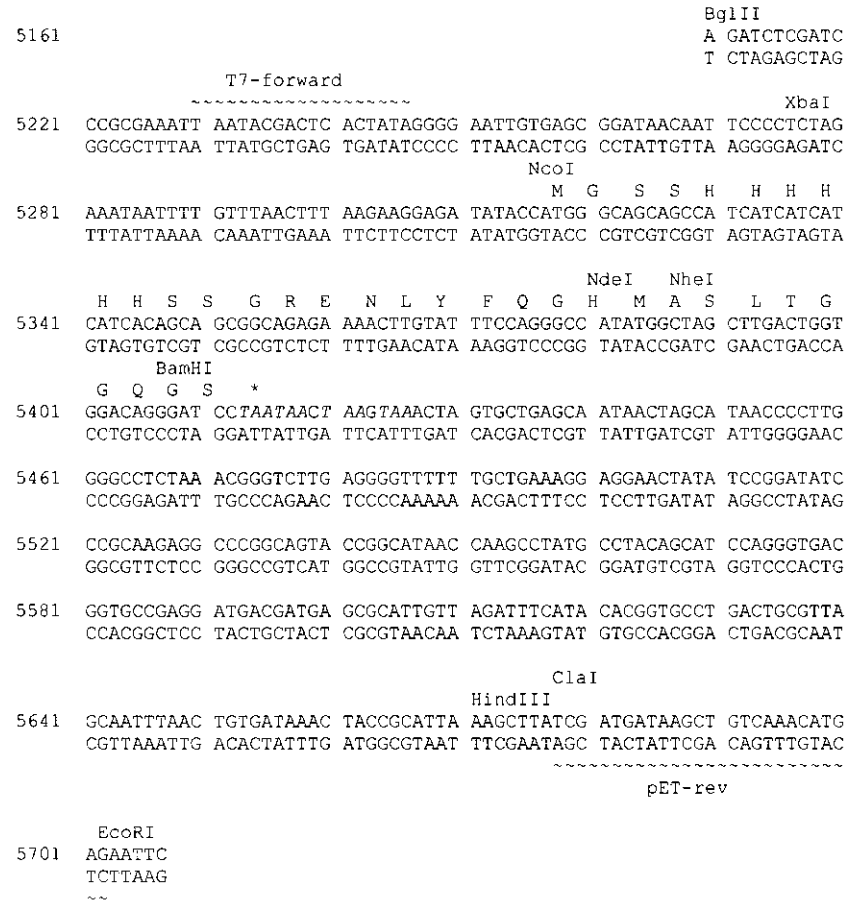
Polylinker region



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pGEX-1λT (27-4805-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Glu Phe Ile Val Thr Asp
CTG GTT CCG CGT GGA TCC CCG GAA TTC ATC GTG ACT GAC TGA CGA

BamH I EcoR I Stop codons

pGEX-2T (27-4801-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Gly Ile His Arg Asp
CTG GTT CCG CGT GGA TCC CCG GGA ATT CAT CGT GAC TGA CTG ACG

BamH I Sma I EcoR I Stop codons

pGEX-2TK (27-4587-01)

Thrombin Kinase

Leu Val Pro Arg↓Gly Ser Arg Arg Ala Ser Val
CTG GTT CCG CGT GGA TCT CGT CGT GCA TCT GTT GGA TCC CCG GGA ATT CAT CGT GAC TGA

BamH I Sma I EcoR I Stop codons

pGEX-4T-1 (27-4580-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Glu Phe Pro Gly Arg Leu Glu Arg Pro His Arg Asp
CTG GTT CCG CGT GGA TCC CCG GAA TTC CCG GGT CGA CTC GAG CGG CCG CAT CGT GAC TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-4T-2 (27-4581-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Gly Ile Pro Gly Ser Thr Arg Ala Ala Ala Ser
CTG GTT CCG CGT GGA TCC CCA GGA ATT CCC GGG TCG ACT CGA GCG GCC GCA TCG TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codon

pGEX-4T-3 (27-4583-01)

Thrombin

Leu Val Pro Arg↓Gly Ser Pro Asn Ser Arg Val Asp Ser Ser Gly Arg Ile Val Thr Asp
CTG GTT CCG CGT GGA TCC CCG AAT TCC CGG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-3X (27-4803-01)

Factor Xa

Ile Glu Gly Arg↓Gly Ile Pro Gly Asn Ser Ser
ATC GAA GGT CGT GGG ATC CCC GGG AAT TCA TCG TGA CTG ACT GAC

BamH I Sma I EcoR I Stop codons

pGEX-5X-1 (27-4584-01)

Factor Xa

Ile Glu Gly Arg↓Gly Ile Pro Glu Phe Pro Gly Arg Leu Glu Arg Pro His Arg Asp
ATC GAA GGT CGT GGG ATC CCC GAA TTC CCG GGT CGA CTC GAG CGG CCG CAT CGT GAC TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-5X-2 (27-4585-01)

Factor Xa

Ile Glu Gly Arg↓Gly Ile Pro Gly Ile Pro Gly Ser Thr Arg Ala Ala Ala Ser
ATC GAA GGT CGT GGG ATC CCC GGA ATT CCC GGG TCG ACT CGA GCG GCC GCA TCG TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codon

pGEX-5X-3 (27-4586-01)

Factor Xa

Ile Glu Gly Arg↓Gly Ile Pro Arg Asn Ser Arg Val Asp Ser Ser Gly Arg Ile Val Thr Asp
ATC GAA GGT CGT GGG ATC CCC AGG AAT TCC CGG GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA

BamH I EcoR I Sma I Sal I Xho I Not I Stop codons

pGEX-6P-1 (27-4597-01)

PreScission™ Protease

Leu Glu Val Leu Phe Gln↓Gly Pro Leu Gly Ser Pro Glu Phe Pro Gly Arg Leu Glu Arg Pro His
CTG GAA GTT CTG TTC CAG GGG CCC CTG GGA TCC CCG GAA TTC CCG GGT CGA CTC GAG CGG CCG CAT

BamH I EcoR I Sma I Sal I Xho I Not I

pGEX-6P-2 (27-4598-01)

PreScission™ Protease

Leu Glu Val Leu Phe Gln↓Gly Pro Leu Gly Ser Pro Gly Ile Pro Gly Ser Thr Arg Ala Ala Ala Ser
CTG GAA GTT CTG TTC CAG GGG CCC CTG GGA TCC CCA GGA ATT CCC GGG TCG ACT CGA GCG GCC GCA TCG

BamH I EcoR I Sma I Sal I Xho I Not I

pGEX-6P-3 (27-4599-01)

PreScission™ Protease

Leu Glu Val Leu Phe Gln↓Gly Pro Leu Gly Ser Pro Asn Ser Arg Val Asp Ser Ser Gly Arg
CTG GAA GTT CTG TTC CAG GGG CCC CTG GGA TCC CCG AAT TCC CGG GTC GAC TCG AGC GGC CGC

BamH I EcoR I Sma I Sal I Xho I Not I