THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 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 ~~also~~ 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	DR. MARC TINI
SIGNATURE	
DEPARTMENT	PHYSIOLOGY AND PHARMACOLOGY
ADDRESS	SIEBENS-DRAKE MEDICAL RES. INSTITUTE, RM 237
PHONE NUMBER	850-2942
EMERGENCY PHONE NUMBER(S)	(519) 694-9102
EMAIL	MTINI@UWO.CA

Location of experimental work to be carried out: Building(s) SDMRI Room(s) 237, 238

*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: CANADIAN CANCER SOCIETY
GRANT TITLE(S): MOLECULAR ANALYSIS OF NOVEL MECHANISMS REGULATING MAMMALIAN GENOME STABILITY AND GENE EXPRESSION

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.

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

RYAN MCHAIN
DEBBIE DONG

* 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 microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒ YES ○ NO
If no, please proceed to Section 2.0

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:

	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
1.	REPLICATION DEFECTIVE	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.01 0L 1X10⁶/mL	PRODUCED IN LAB	○ 1 ☒ 2 ○ 3
	MALONEY MURINE LEUKEMIA VIRUS	○ Yes ☒ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3
2.	LABORATORY ESCHERICHA COLI	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.5 L 1X10⁹ cfu/mL	PROMEGA	☒ 1 ○ 2 ○ 3
3.	BACULOVIRUS (INSECT VIRUS)	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.050L 3X10⁷ pfu/mL	PRODUCED IN LAB	☒ 1 ○ 2 ○ 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? ☒ 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 in the table below

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	MOUSE EMBRYO FIBROBLASTS	2006-086-08
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 the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	HELA, HELAS3, MCF7	ATCC
Rodent	☒ Yes ○ No	NIH3T3	ATCC
Non-human primate	☒ Yes ○ No	COS7	ATCC
Other (specify)	☒ Yes ○ No	SF9 (INSECT)	ATCC

*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 ○ 3

3.0 Use of Human Source Materials

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

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be 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		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ☒ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)	BRAIN TUMOUR TISSUE BANK	○ Yes ☒ No		☒ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)	ONTARIO TUMOUR BANK	○ Yes ☒ No		☒ 1 ○ 2 ○ 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5X, BL21 E. coli	PCMX, PET PLNEX1 PGEX	CLONTECH NOVAGEN GE LIFE SCIENCES	THYMINE DNA GLYCOSYLASE AND ASSOCIATED TRANSCRIPTION FACTORS	POTENTIAL CYTOTOXICITY DUE TO EXPRESSION OF MAMMALIAN PROTEIN

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

4.3 Will genetic modification(s) involving viral vectors be done? ☒ YES, complete table below ○ NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
MALONEY MURINE LEUKEMIA VIRUS	PLNCX	CLONTECH	THYMINE DNA GLYCOSYLASE	POTENTIAL CELL CYCLE AND DNA REPAIR DEFECTS

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ☒ YES ○ NO

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

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

5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? ○ YES, number: _____ ○ NO ○ PENDING

6.0 Animal Experiments

6.1 Will live animals be used? ○ 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 be used in live animals ○ YES, specify: _____ ○ NO

7.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs O YES ☒ NO
- Pound source cats O YES ☒ NO
- Cattle, sheep or goats ☒ YES O 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*? _____

*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 lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" 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 X 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

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 10.0 X NO

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

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES X 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. O 1 X 2 O 3

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

X YES, permit # if on-campus BIO-UWO-0151

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: 11/11/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

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

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

Special Conditions of Approval:

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

Subject: RE: Biohazardous Agents Registry Form: Tini
From: Marc Tini <mtini@uwo.ca>
Date: Tue, 26 May 2009 15:46:30 -0400
To: 'Jennifer Stanley' <jstanle2@uwo.ca>

Hello Jennifer,

In Section 7.1 I am referring to fetal bovine serum used in cell culture media.
We do not perform any pseudotyping of virus. We produce ecotrophic and amphotropic virus using the Phoenix packaging cell lines (http://www.stanford.edu/group/nolan/tutorials/retpkg_7_phx_sys.html).

Regards,
Marc

Marc Tini, PhD
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http://publish.uwo.ca/~mtini/

-----Original Message-----
From: Jennifer Stanley [mailto:jstanle2@uwo.ca]
Sent: May 26, 2009 3:03 PM
To: Marc Tini
Subject: Biohazardous Agents Registry Form: Tini

Dr. Tini
The Biohazards Subcommittee recently reviewed your project (see attached).
Please let us know the following questions:
1. What is the goat/sheep/cattle product you use in Section 7.1?
2. Is there any pseudotyping of virus done or is it ecotropic?
Thanks
Jennifer

In vertebrate genomes the cytosine-guanine (CpG) dinucleotides mutate at a high rate due to spontaneous deamination of cytosine which results in CpGs occurring at only one quarter of the expected frequency. The CpG mutation rate is increased by methylation of cytosine, which promotes deamination to form thymine. Consequently, in humans the incidence of 5-methylcytotosine to thymine mutations is 10 to 50-fold higher than other base transitions. As a result, more than one third of germline point mutations in genetic diseases and a large fraction of somatic mutations in cancer are due to CpG hypermutability. Cytosine methylation also has an integral role in transcriptional regulation and the promoter regions of genes are enriched in CpG. The integrity of CpGs in these regions is safeguarded by thymine DNA glycosylase which removes aberrant thymine and uracil from G:T and G:U mispairs produced by deamination of 5-methylcytosine and cytosine, respectively. TDG also functionally interacts with transcription factors and epigenetic regulators and serves as a transcriptional cofactor. In previous studies, we have shown that TDG associates directly with protein acetylases CBP (CREB-binding protein) and p300, and the resulting complexes participate in both DNA repair and transcriptional regulation. TDG strongly activates CBP/p300-dependent transcription and reciprocally serves as a substrate for CBP/p300 acetylation. We have shown that two Small Ubiquitin-like Modifier-1 (SUMO-1) protein-binding motifs in TDG are essential for transcriptional stimulation and regulation of subnuclear localization. Moreover, sumoylation of TDG abrogates DNA binding and CBP/p300 association. Recently, our studies have revealed that protein kinase C α (PKCα) interacts with the amino-terminal region of TDG and phosphorylates serine residues adjacent to lysines acetylated by CBP/p300. We found that acetylation and phosphorylation are mutually exclusive and their interplay dramatically alters the DNA mispair processing functions. Remarkably, acetylation selectively prevents G:T processing by abrogating DNA interactions. In contrast, phosphorylation does not alter substrate interactions but may preserve G:T processing *in vivo* by preventing CBP-mediated acetylation. These findings highlight the importance of posttranslational modifications (PTMs) in regulating TDG and suggest that their interplay *in vivo* is critically important in CpG maintenance and epigenetic regulation. Consistent with the important roles of TDG, we have demonstrated that disruption of the *Tdg* gene causes embryonic lethality in mice. In view of the instability of CpG dinucleotides, and the link between aberrant genomic methylation and cancer, elucidation of the physiological roles and regulation of TDG will further our understanding of the molecular processes deregulated in cancer cells. I plan to further investigate the biological functions of TDG by pursuing the following objectives.

Aim I. Molecular analysis of the genome maintenance and gene regulatory functions of TDG: To further elucidate the genome surveillance and transcriptional roles of TDG, we will map the *in vivo* binding sites in the entire genome using chromatin immunoprecipitation on chip (ChIP-chip) assays. We will also use ChIP assays to decipher the mechanisms that target TDG to promoters and determine the role of PTMs in regulating the CpG maintenance and transcriptional functions.

Aim II. Characterization of TDG posttranslational modifications in normal and transformed cells: We will employ PTM-specific antibodies and 2-D polyacrylamide gel electrophoresis to investigate the physiological context for these modifications and establish whether alterations in PTMs are associated with different types of human cancer.

Aim III. Elucidation of the developmental and physiological roles of TDG: We will continue our analysis of *Tdg* null mice and identify potential causes of embryonic lethality by characterizing tissue, cellular and gene expression defects. Additionally, to permit the analysis of cancer susceptibility resulting from TDG deficiency, we will generate mice with a conditional *Tdg* allele that can be ablated in a temporal/spatial specific manner.

These investigations will contribute to a better understanding of the molecular events that regulate genome stability and should uncover new molecular tools for the diagnostic and therapeutic management of cancer.

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: CCL-2™ Order this Item
Designations: HeLa
Biosafety Level: 2 [CELLS CONTAIN PAPOVAVIRUS]
Medium & Serum: See Propagation
Organism: *Homo sapiens* (human)

Price: $256.00
Depositors: WF Scherer
Shipped: frozen
Growth Properties: adherent
Morphology: epithelial

Source: **Organ:** cervix
Disease: adenocarcinoma
Cell Type: epithelial

Cellular Products: keratin
Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-Jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [20623]

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

Applications: transfection host ([21491] Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
screening for Escherichia coli strains with invasive potential [21447] [21491]

Virus Susceptibility: Human adenovirus 3
Encephalomyocarditis virus
Human poliovirus 1
Human poliovirus 2
Human poliovirus 3

Reverse Transcript: negative

DNA Profile (STR): Amelogenin: X
CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10
D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

Cytogenetic Analysis: Modal number = 82; range = 70 to 164.
There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of M1, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.

Isoenzymes: G6PD, A

Age: 31 years adult

Gender: female

Ethnicity: Black

HeLa Markers: Y

Comments: The cells are positive for keratin by immunoperoxidase staining.
HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences.
P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
derivative:ATCC CCL-2.3
derivative:ATCC CCL-2.1
derivative:ATCC CCL-2.2

Bioreactive Factors: **Growth Factors:** T cell growth factor (TCGF)

References:

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28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321

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Cell Biology

ATCC® Number:	**CCL-2.2™** Order this Item	**Price:**	**$268.00**
Designations:	HeLa S3	**Depositors:**	TT Puck
Biosafety Level:	2 [Cells contain human papilloma virus (HPV-18)]	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
Source:	**Organ:** cervix **Disease:** adenocarcinoma		
Cellular Products:	keratin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location. ***Related Cell Culture Products***		
Isolation:	**Isolation date:** 1955		
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from cattle)		
Virus Susceptibility:	poliovirus 1, 2, 3; vesicular stomatitis (Indiana); encephalomyocarditis; adenovirus 5		
Reverse Transcript:	negative		
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 13,3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18		
Cytogenetic Analysis:	A medium-sized metacentric marker is present in 100% of the cells. HeLa Markers: One copy of M1, one copy of M2, two copies of M3, and one copy of M4.		
Isoenzymes:	G6PD, A		
Age:	31 years		
Gender:	female		
Ethnicity:	Black		
HeLa Markers:	Y		
Comments:	HeLa S3 is a clonal derivative of the parent HeLa line (see ATCC CCL-2). S3 was cloned in 1955 by T.T. Puck, P.I Marcus, and S.J. Cieciura. [22514] The HeLa S3 clone has been very useful in the clonal analysis of mammalian cell populations relating to chromosomal variation, cell nutrition, and plaque-forming ability. This line can be adapted to grow in suspension. [25952] The cells are positive for keratin by immunoperoxidase staining. A culture at approximately passage 400 was submitted to the American Type Culture Collection in February, 1972 HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. [23180]		

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Cell Biology

ATCC® Number: HTB-22™ Order this Item
Designations: MCF7
Biosafety Level: 1
Medium & Serum: See Propagation
Organism: *Homo sapiens* (human)

Price: $264.00
Depositors: CM McGrath
Shipped: frozen
Growth Properties: adherent
Morphology: epithelial

PHOTO

Source:
Organ: mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

Cellular Products: insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

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

Receptors: estrogen receptor, expressed

Antigen Expression: Blood Type O; Rh+

DNA Profile (STR):
Amelogenin: X
CSF1PO: 10
D13S317: 11
D16S539: 11,12
D5S818: 11,12
D7S820: 8,9
THO1: 6
TPOX: 9,12
vWA: 14,15

Cytogenetic Analysis: modal number = 82; range = 66 to 87.
The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.

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

Age: 69 years adult

Gender: female

Ethnicity: Caucasian

Comments: The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088].
Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha).
Secretion of IGFBP's can be modulated by treatment with anti-estrogens.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10% .
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.
Note: if floating cells are present, it is recommended that they be transferred at the first two (2) subcultures as described below. It is not necessary to transfer floating cells for subsequent subcultures.

1. Remove culture medium to a centrifuge tube.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer the cell suspension to the centrifuge tube with the medium and cells from step 1, and centrifuge at approximately 125 xg for 5 to 10 minutes. Discard the supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels.
7. Incubate cultures at 37C.

Subcultivation Ratio: A subcultivation ratio of 1:3 to 1:6 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 29 hrs

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
recommended serum:ATCC 30-2020
purified DNA:ATCC HTB-22D
purified RNA:ATCC HTB-22R
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X

References:
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22871: Takahashi K, Suzuki K. Association of insulin-like growth-factor-I-induced DNA synthesis with phosphorylation and nuclear exclusion of p53 in human breast cancer MCF-7 cells. Int. J. Cancer 55: 453-458, 1993. PubMed: 8375929
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Cell Biology

ATCC® Number: CRL-1658™ Order this Item
Designations: NIH/3T3
Biosafety Level: 1
Medium & Serum: See Propagation
Organism: *Mus musculus* (mouse)

Price: $256.00
Shipped: frozen
Growth Properties: adherent
Morphology: fibroblast

PHOTO

Source:
Organ: embryo
Strain: NIH/Swiss
Cell Type: fibroblast fibroblast;

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

Related Cell Culture Products

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

Virus Susceptibility: Murine leukemia virus

Age: embryo

Comments: The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457].
Tested and found negative for ectromelia virus (mousepox).

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less.
Subcultivation Ratio: Inoculate 3 to 5 X 10(3) cells/cm2
Medium Renewal: Twice per week

Preservation:
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:
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Cell Biology

ATCC® Number: CRL-1651™ Order this Item
Designations: COS-7
Biosafety Level: 2 [Cells Contain SV-40 viral DNA sequences]
Medium & Serum: See Propagation
Organism: *Cercopithecus aethiops*

Price: $264.00
Depositors: Y Gluzman
Shipped: frozen
Growth Properties: adherent
Morphology: fibroblast

Source: **Organ:** kidney
Cell Type: SV40 transformed
Cellular Products: T antigen

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Related Cell Culture Products

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE ® Transfection Reagents)
Virus Susceptibility: SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region

Comments: This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.

Propagation: **ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing: **Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:
0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101
Cell culture tested DMSO:ATCC 4-X
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020
parental cell line:ATCC CCL-70

References:
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Cell Biology

ATCC® Number: CRL-1711™ Order this Item
Designations: Sf9
Biosafety Level: 1
Medium & Serum: See Propagation
Organism: Spodoptera frugiperda (fall armyworm)

Price: $264.00
Depositors: G Smith, C Cherry, MD Summers
Shipped: frozen
Growth Properties: mixed: adherent/suspension
Morphology: epithelial

PHOTO

Source: **Organ:** ovary

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Related Cell Culture Products

Isolation: **Isolation date:** 1983

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

Virus Susceptibility: Nuclear polyhedrosis virus, Autographa californica
St. Louis encephalitis virus

Age: pupa

Gender: female

Comments: This line can be used to replicate baculovirus expression vectors.
It is important to use the medium described below.

Propagation: **ATCC complete growth medium:** Grace's Insect Medium with L-glutamine and supplemented with:
500 mg/L calcium chloride
2800 mg/L potassium chloride
3330 mg/L lactalbumin hydrolysate
3330 mg/L yeastolate
10% Heat-inactivated fetal bovine serum (previously tested for insect cell culture)
Atmosphere: air, 100%
Temperature: 28.0°C
Growth Conditions: The recommended media are formulated for use without CO2. Omission of the yeastolate or lactalbumin hydrolysate will lead to poor performance by this line.

Subculturing: **Protocol:** Gently resuspend cells in the spent culture medium by pipetting across the monolayer or by hitting the flask against the palm of your hand (the latter is only preferable when working with larger flasks).
Subcultivation Ratio: A subcultivation ratio of 1:5 or greater is recommended
Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** culture medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

References:
22650: Vaughn JL, et al. The establishment of two cell lines from the insect Spodoptera frugiperda (Lepidoptera; Noctuidae). In Vitro 13: 213-217, 1977. PubMed: 68913
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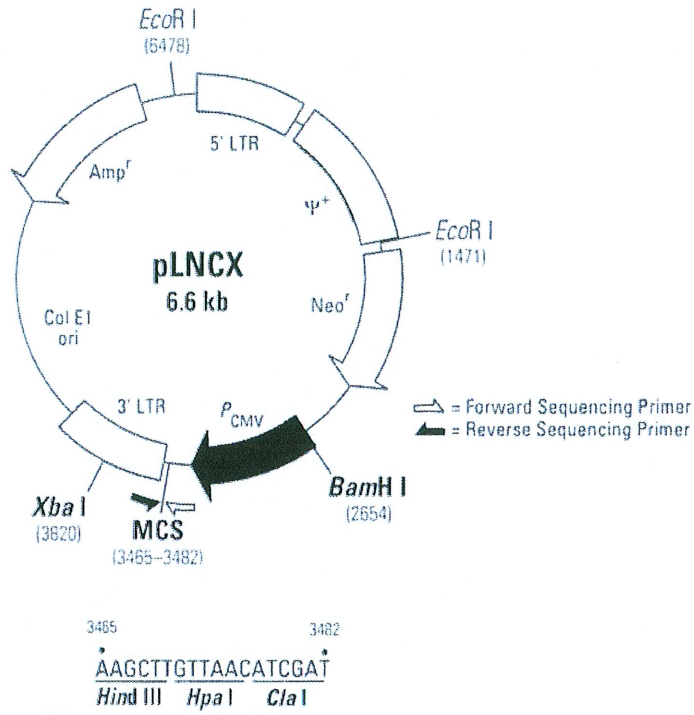

Restriction Map and Multiple Cloning Site (MCS) of pLNCX Retroviral Vector. Unique restriction sites are in bold.

Description

pLNCX contains elements derived from Moloney murine leukemia virus (MoMuLV) and Moloney murine sarcoma virus (MoMuSV), and is designed for retroviral gene delivery and expression (1–3). Upon transfection into a packaging cell line, pLNCX can transiently express, or integrate and stably express, a transcript containing Ψ+ (the extended viral packaging signal) a selectable marker, and the gene of interest. The 5' viral LTR in this vector contains promoter/enhancer sequences that control expression of the neomycin resistance (Neo^r) gene for antibiotic selection in eukaryotic cells. A gene of interest can be cloned into the multiple cloning site immediately downstream of the human cytomegalovirus (CMV) immediate early promoter (P_{CMV}). pLNCX also includes the Col E1 origin of replication and *E. coli* Amp^r gene for propagation and antibiotic selection in bacteria.

Use

As a part of the Retro-X™ System (#K1060-1), pLNCX can be transfected into a packaging cell line such as the RetroPack™ PT67 Cell Line. Once in the cell, RNA from the vector is packaged into infectious, replication-incompetent retroviral particles. pLNCX does not contain the structural genes (*gag*, *pol*, and *env*) necessary for particle formation and replication, however, these genes are stably integrated into PT67 (4–7). Subsequent introduction of pLNCX, containing Ψ+ (psi), transcription and processing elements, and the gene of interest produces high-titer, replication-incompetent infectious virus. That is, these retroviral particles can infect target cells and transmit the gene of interest (which is cloned between the viral LTR sequences), but cannot replicate within these cells since the cells lack the viral structural genes. The separate introduction and integration of the structural genes into the packaging cell line minimizes the chances of producing replication-competent virus due to recombination events during cell proliferation.

(PR99507, published 17 January 2000)

Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Neomycin resistance gene (Neo^r):
  Start codon: 1512–1514; stop codon: 2304–2306
- Immediate early CMV promoter (P_{CMV}): 2656–3393
- Multiple Cloning Site (MCS): 3465–3482
- 3' MoMuLV LTR: 3522–4115
- Col E1 origin of replication:
  Site of replication initiation: 4651
- Ampicillin resistance gene (β-lactamase):
  Start codon: 6271–6269; stop codon: 5413–5411

Sequencing Primer Locations

- pLNCX Seq/PCR Primers (#K1060-F)
  5' primer (3368–3393): 5'-AGCTCGTTTAGTGAACCGTCAGATCG-3'
  3' primer (3544–3519): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: low

References

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6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
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Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by CLONTECH. This vector has not been completely sequenced.

- Technical Resources
- Technical Information
- Novagen Information
- Vector Tables
  - BlueStar Vectors
  - Duet Vectors
  - Gateway® Nova DEST Vectors
  - LIC Duet Adaptors
  - M13mp18 Vector
  - pBAC Vectors
  - pBiEx Vectors
  - pCDF & pRSF Vectors
  - pCITE Vectors
  - **pET Vectors**
  - pETBlue Vectors
  - pETcoco Vectors
  - pIEx Vectors
  - pIEx/Bac Vectors
  - pT7Blue Vectors
  - pTandem Vectors
  - pTriEx Vectors
  - SCREEN Vectors
  - T7Select Vectors
  - Discontinued Vectors

pET *E. coli* T7 expression vectors

The pET System is the most powerful system yet developed for the cloning and expression of recombinant proteins in *E. coli*. Based on the T7 promoter-driven system originally developed by Studier and colleagues (1–3), Novagen's pET System has been used to express thousands of different proteins. In pET vectors, target genes are cloned under control of strong bacteriophage T7 transcription and translation signals, and expression is induced by providing a source of T7 RNA polymerase in the host cell.

Novagen's pET System has continuously expanded to offer new technologies and options for expression, and includes over 42 pET vector types, 15 different host strains and many other companion products designed for efficient detection and purification of target proteins.

1. Moffatt, B.A. and Studier, F.W. (1986) *J. Mol. Biol.* **189**, 113–130.
2. Rosenberg, A.H., Lade, B.N., Chui, D., Lin, S., Dunn, J.J., and Studier, F.W. (1987) *Gene* **56**, 125–135.
3. Studier, F.W., Rosenberg, A.H., Dunn, J.J., and Dubendorff, J.W. (1990) *Meth. Enzymol.* **185**, 60–89.

pET Vectors with unique features include:

- pET-31b(+) for high yield bioproduction of peptides and small proteins
- pET-32a-c for production of soluble, active target proteins in *E. coli*
- pET-33b for production of target proteins suitable for site-specific ^{32}P-labeling
- pET-39b and 40b using Dsb tags for export and periplasmic folding of target proteins
- pET-41a-c and 42a-c with popular GST fusion tags for enhanced production and solubility
- pET-43.1a-c designed for cloning and high-level expression of polypeptide sequences fused with the 495 aa NusA (Nus•Tag™) protein
- pET-44a-c with Nus•Tag™ sequence plus N- and C-terminal His•Tag sequences
- pET-45b(+) with amino-terminal His•Tag™ sequence and minimal extraneous sequences
- pET-46 Ek/LIC prepared vector for ligation-independent cloning, with amino-terminal His•Tag™ sequence
- pET-47b(+) through pET-50b(+) with HRV 3C Protease cleavage site for efficient fusion tag removal

Additional vectors in this table include:

- pLacI
- pLysE and pLysS

A Note on the Sequences

The numbering system of the pET vectors is based on that of pBR322, from which all of these vectors are derived. The sequences are given in the same orientation as the pBR322 sequence, but note that this is the **opposite** orientation of the the cloning/expression region detail maps. This should be apparent from the counter-clockwise orientation of the T7 promoter on the circle maps.

Most of the vector sequences are provided in GenBank format, with significant features indicated. To convert the sequence into other formats, first select the sequence and copy (Command-C on Mac or Ctrl-C on Windows). Next, click here to open the NIH ReadSeq web page in a new window. Paste the sequence into the input field (Command-V on Mac or Ctrl-V on PC), choose your favorite format from the dropdown menu, and hit the "submit" button.

You can access sequences, maps, ordering information, newsletter articles and technical bulletins through the following links:

New-style table Leave feedback on the new style!

Vector	Sequence	Map	Ordering Information	Newsletter Articles	Technical Bulletins
pET-3a					
pET-3b					

pGEX Vectors (GST Gene Fusion System)

Technical Information

Thirteen pGEX vectors are available (see figure). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors. pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3 each encode the recognition sequence for site-specific cleavage by PreScission™ Protease, (see PreScission Protease) between the GST domain and the multiple cloning site. pGEX-4T-1, pGEX-4T-2, and pGEX-4T-3 are derived from pGEX-2T and contain a thrombin protease recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX5X-3 are derivatives of pGEX-3X and possess a factor Xa protease recognition site.

Download the pGEX sequence map in PDF format. For ASCII format please scoll down.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labeling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from heart muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labeled using protein kinase and [γ-^{32}P]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T, its fusion proteins can be cleaved with thrombin.

Cleavage of pGEX-6P GST fusion proteins occurs between the Gln and Gly residues of the recognition sequence Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro [2]. Low temperature (5°C) digestion minimizes the degradation of the protein of interest. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed from the cleavage mixture simultaneously with the GST portion of the fusion protein. The pGEX-6P Expression Vectors permit convenient site-specific cleavage and simultaneous purification on Glutathione Sepharose™. The pGEX-6P series provides all three translational reading frames linked between the GST coding region and the multiple cloning site.

Collectively, the pGEX vectors provide all three translational reading frames beginning with the EcoR I restriction site. pGEX-1lT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from l gt11 libraries.

Vector	Unformatted	Formatted	GenBank Accession No.	
pGEX-1 lambda T, 27-4805-01	*ASCII*	*PDF*	U13849	
pGEX-2T, 27-4801-01	*ASCII*	*PDF*	U13850	
pGEX-2TK, 27-4587-01	*ASCII*	*PDF*	U13851	
pGEX-3X, 27-4803-01	*ASCII*	*PDF*	U13852	
pGEX-4T-1, 27-4580-01	*ASCII*	*PDF*	U13853	
pGEX-4T-2, 27-4581-01	*ASCII*	*PDF*	*PDF*	U13855
pGEX-5X-1, 27-4584-01	*ASCII*	*PDF*	U13856	
pGEX-5X-2, 27-4585-01	*ASCII*	*PDF*	U13857	
pGEX-5X-3, 27-4586-01	*ASCII*	*PDF*	U13858	
pGEX-6P-1, 27-4597-01	*ASCII*	*PDF*	U78872	
pGEX-6P-2, 27-4598-01	*ASCII*	*PDF*	U78873	
pGEX-6P-3, 27-4599-01	*ASCII*	*PDF*	U78874	

Click on "ASCII" to download an unformatted sequence for use by a sequence analysis program. Click on "PDF" to download a formatted sequence and restriction site table. If you prefer accessing the sequence in *GenBank*, refer to the right-hand column for the GenBank accession number:

- **Expression:** Proteins are expressed as fusion proteins with the 26 kDa glutathione S-transferase (GST). The GST gene contains an ATG and ribosome-binding site, and is under control of the tac promoter. A translation terminator is provided in each reading frame. The resulting fusion protein may be purified using the GST Purification Module (27-4570-01, -02; see GST Purification Modules.)
- **Enzymatic cleavage with PreScission™ Protease:** pGEX-6P-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with PreScission™ Protease. Because PreScission™ Protease has been engineered with a GST-tag, it can also be removed simultaneously with the GST portion of the fusion protein.
- **Enzymatic cleavage with thrombin:** pGEX-1 lambda T, pGEX-2T, pGEX-2TK, pGEX-4T-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with thrombin.
- **Enzymatic cleavage with factor Xa:** pGEX-3X, pGEX-5X-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with factor Xa.
- **Direct labeling *in vitro*:** pGEX-2TK allows for direct labeling of fusion proteins in vitro with 32P using the catalytic subunit of cAMP-dependent protein kinase.
- **Host(s):** *E. coli*. The plasmid provides lac Iq repressor.
- **Selectable marker(s):** Plasmid confers resistance to 100 µg/ml ampicillin.
- **Amplification:** Recommended.

Properties of pGEX Vectors • Induction: tac promoter inducible with 1-5 mM IPTG.