THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
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).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) 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

PRINCIPAL INVESTIGATOR	Joseph Torchia
DEPARTMENT	Oncology, Cancer Research Laboratories, LRCP
ADDRESS	790 Commissioners Rd. E., Room A4-915
PHONE NUMBER	519-685-8692
EMERGENCY PHONE NUMBER(S)	
EMAIL	jtorchia@uwo.ca

Location of experimental work to be carried out: Building(s): LRCP Room(s) A4-915, A4-910, A4-911, A4-917

*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: Canadian Institute of Cancer Research (CIHR)
GRANT TITLE(S): The role of the ZNF217 oncoprotein in gene regulation and cancer

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

Name	UWO E-mail Address	Date of Biosafety Training
Majdina Isovic	mbambego@uwo.ca	August 3, 2010
Gobi Thillainadesan	gthillai@uwo.ca	September 2006
Niamh Coughlan	ncoughla@uwo.ca	September 18, 2007
Bartlomiej Kolendowski	bkolendo@uwo.ca	September 2011

Changes to Entire Form

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.
Our laboratory is certified as Level 2 lab under Biosafety Approval Number BIO-LRCC-0005.

Biological Agent: Spontaneously Immortalized cell lines and Human cancer cell lines (list attached)
Usage: Live cells maintained in cell culture incubator and handled in laminar flowhood BSC. Cell lines listed under section 2.3 have all been obtained through a commercial supplier (see attached MSDS). The only modifications made to these cell lines are by transfection of the plasmids (see below) so that the cell line would overexpress tagged, mutated or truncated proteins allowing us to study the importance of the proteins in question.
Storage: Stored in freezer bank (-80 and -150 freezers)
Disposal: Liquids that contain cells are bleached and flushed down the drain
Plasticware that has been in contact with cells is disposed into biohazard waste boxes (lined with a yellow bag) and disposed by licensed waste carrier

Biological Agent: Adenovirus
Usage: virus is handled in Level II biohazard facility. In a laminar flow hood BSC, and maintained in a dedicated cell culture incubator. Users must wear PPE and all materials that contacts the virus must thoroughly decontaminate prior to disposal or transfer to another facility. The adenovirus is already in the lab and was obtained from Dr. Paul Yaswens lab at the University of California at Berkeley. The adenovirus contains ZNF217 cDNA, a putative oncogene. The ZNF217 adenovirus will be used to transduce the human HaCAT cell line, a nontransformed keratinocyte cell line in culture. **However, in this vector early Regions 1 (E1) and 3 (E3) of wild-type adenovirus have been deleted. Deleting E1 restricts the cytopathic activity of the recombinant adenoviral particles produced. This safety feature means that replication-incompetent adenovirus are produced, which propagates only in those cell types (e.g., HEK 293 cells) that express the E1-encoded factors. For all other somatic cell types susceptible to adenoviral infection, exposure to the recombinant adenovirus leads to a transient non-cytopathic (i.e., non-lytic) infection. The adenoviral genome is established as an episome in the host cell's nucleus, but is neither replicated nor *actively* transcribed since the cell lacks the necessary transcription factors—the E1 gene products. However, ZNF217 is still expressed at high levels because it is independently controlled by the constitutive cytomegalovirus (CMV) promoter. Expression of ZNF217 depends neither on the proliferation of the target cell line nor on the presence of any other viral genes or promoters. Expression is transient because adenoviral DNA does not integrate into the cellular genome. Finally, deletion of E3 region means that that this virus does not express the E3 14.7K, 14.5K and 10.4K proteins which normally block efficient evasion of the host immune response. The National Institute of Health and Center for Disease Control have designated adenoviruses as Level 2 biological agents. This distinction requires the maintenance of a Biosafety Level 2 facility for work involving this virus.**
Storage: virus kept in culture medium in -80 freezer
Disposal: virus is thoroughly bleached prior to flushing down the drain
Plasticware that has contacted virus is bleached, then autoclaved (located on the floor) immediately prior to disposal in a biohazardous waste container
The operational guidelines are in compliance with the university guidelines described in the document located at :
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/viral_vector_policy.pdf

Biological Agent: Baculovirus
Usage: virus is handled in Level II biohazard facility. In a laminar flow hood BSC, and maintained in a dedicated cell culture incubator. Users must wear PPE and all materials that contacts the virus must thoroughly decontaminate prior to disposal or transfer to another facility. Baculoviruses are generated in my laboratory using the *bac-to-bac* baculvirus kit obtained from Invitrogen. The baculoviruses are used to infect insect cells (SF9 or SF21) and are used to study protein interactions, or for protein expression.
Storage: Stored at 4C in a designated area.
Disposal: Liquids that contain cells are bleached and flushed down the drain. Plasticware that has been in contact with cells is disposed into biohazard waste boxes (lined with a yellow bag) and disposed by licensed waste carrier

Biological Agent: Bacterial cells
Usage: For cloning applications we use the DH5α strain of E.coli, purchased from Invitrogen. Any liquid waste generated by growing bacterial cultures is bleached and subsequently disposed of. Solid waste is directly disposed of in the biohazardous waste. For generation of baculovirus, the DH10bac cell line is specifically used and for protein expression we use the BL21 bacterial cell line.
Disposal: Liquids that contain cells are bleached and flushed down the drain. Plasticware that has been in contact with cells is disposed into biohazard waste boxes (lined with a yellow bag) and disposed by licensed waste carrier, Stericycle (autoclaved and/or incinerated before disposing in landfill).

Biological Agent: Plasmids
Usage: Plasmids used for cloning have originally been purchased from different suppliers (please see attached information). All of the plasmid vectors we are using are well characterized and either are non-mobilisable (eg pBKS/PUC series and their derivatives) or mobilisation-defective (eg pCDNA3/pBR322 and its derivatives) in bacteria. They contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no harmful sequences. Some vectors may include sequences derived from mammalian (CMV) or insect viruses for the purpose of achieving a specific fate upon subsequent transfection of eukaryotic cells. These are generally restricted to well-characterized sequences that are completely harmless in isolation from other viral sequences. Some modifications have been made to the inserts and at present our laboratory has 1200+ different constructs. The newly generated plasmids either have different C or N-terminal tags added to the cDNA of interest to allow distinction from the native protein for overexpression studies. Commonly we also make truncations to a cDNA of interest and clone into different plasmid backbones (application dependant transient transfection, protein expression) so that different functional protein regions can be identified. We often resort to mutational studies where binding or interaction sites are mutated resulting in downstream effects.

The two projects in the lab are:

1.The role of the ZNF217 oncoprotein in gene regulation and cancer
Cancer is a disease involving aberrant cell growth in which the normal control of cell proliferation and division are disrupted. Many proteins that give rise to cancer play an essential role in processes that controls the cells ability to undergo cell division and multiplication. However, when these proteins are mutated, inactivated or amplified, normal growth patterns may be disrupted which could have devastating consequences to the organism. The research in my lab is dedicated to identifying and understanding how specific proteins contribute to the development of cancer. ZNF217 is a candidate oncogene that is amplified and overexpressed in multiple cancer cell lines and tumours and recent evidence indicates that ZNF217 overproduction results in abnormal cell growth. The hypothesis behind the proposed research is that deregulated expression of the ZNF217 transcription factor promotes the development of cancer by causing aberrant transcriptional silencing of specific target genes. This hypothesis is based on the following observations: First, ZNF217 is found within a narrow region of recurrent maximal amplification that is devoid of other transcribed genes. Secondly, we and others have shown that ZNF217 is a major constituent of a transcriptional complex containing chromatin-modifying activities known to promote transcriptional repression. Thirdly, we have identified a consensus recognition sequence suggesting that ZNF217 has DNA binding activity. Fourth, we are combining (1) genome wide expression profiling of cancer cells in which ZNF217 has been downregulated using siRNA and (2) chromatin immunoprecipitation (ChIP) in conjunction next generation sequencing, to identify genes directly regulated by ZNF217. Importantly, we have identified and validated the first known targets of the human ZNF217 complex, several of which play a direct role in tumour biology. As part of our long term goals to understand how defects in gene expression pathways contribute to tumourigenesis, the specific objectives of this application are designed to define the role of ZNF217 in transcriptional regulation, as well as explore potential functions that may be relevant to aberrant cell

proliferation and cancer (funded by the CIHR).

2. The role of TGFβ in epigenetic stability
5 methylcytosine (5MeC) serves a fundamental role in gene regulation but also increases the risk of mutations because methylated cytosines have a greater tendency to spontaneously deaminate to thymine generating a G:T mispair. CpG methylation is also important for the establishment of proper chromatin states and DNA methylation patterns (i.e. epigenomic stability); in cancer, normal DNA methylation patterns are disrupted, but the underlying mechanisms are not well understood. Thymine DNA glycosylase (TDG) is a base excision repair enzyme that maintains genomic stability by removing mispaired thymine or uracil in a CpG context. Recent evidence suggests that TDG is also essential for the localized removal of 5MeC (DNA demethylation). We have made the novel discovery that that transforming growth factor beta (TGFβ) activates the p15ink4b tumour suppressor gene by promoting DNA demethylation via recruitment of (TDG) and other base excision repair enzymes. TGFβ regulates a network of genes involved in cell proliferation, differentiation, apoptosis and development and these cellular pathways are often disrupted during tumour progression. As part of our longterm goals to understand how disruption of TGF-beta signalling contributes to cancer progression, we propose to define the epigenetic consequences of TGF-beta signaling (Funded by the Cancer Research Society).

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O 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? O YES X 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 (DH5α & BL21 DH10 Bac)	O Yes X No	O Yes X No	O Yes X No	1Litre	Invitrogen	X 1 O 2 O 2+ O 3
ZNF217 Adenovirus (replication defective)	X Yes O No	X Yes O No	O Yes X No	0.01Litre Titer~10^9 pfu/ml	Dr. Paul Yaswens laboratory U Cal Berkeley (See MSDS from Public Health	O 1 x 2 O 2+ O 3

					agency of Canada	
baculovirus	O Yes X No	O Yes X No	O Yes X No	0.1 Litres Titer~10^9 pfu/ml	Invitrogen	O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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? X 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 X No		Not applicable
Rodent	X Yes O No	Mouse embryos	#2009-052
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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)*	Supplier / Source
Human	x Yes O No	HeLa, MCF7, HaCAT, 293T, HT1080, IMR90, Wi-38,	ATCC/HaCAT cells were obtained from Dr. Lina Dagnino at the UWO.
Rodent	x Yes O No	Mouse embryonic stem cells (MES) / mouse embryonic fibroblasts (MEFs)	ATCC
Non-human primate	O Yes X No		
Other (Insect cells)	X Yes O No	SF9/SF21	Invitrogen

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) **MSDS not available for HaCAT as obtained from Dagnino Lab on campus.**

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

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

3.2 Indicate 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 Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 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? x YES NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5α	The lab has approximately 1200+ different plasmids on hand. Most commonly used backbones are pBKS, pFastbac, pCMX, pcDNA3, pGEX, etc. Backbone used is highly dependant on the application. Transient transfection, stable cell line generation or protein expression.	Original constructs have been purchased from various companies, including Promega, Invitrogen, GE Healthcare, and Stratagene.	ZNf217, Thymine DNA glycosylase(TDG) and other proteins which associate with these two. CtBP1, HDACs, CoREST CBP/p300, SMADs	All of the vectors we are using are well characterized are either non-mobilisable (eg pBKS/PUC series and their derivatives) or mobilisation-defective (eg pCDNA3/pBR322 and its derivatives) in bacteria. In addition they contain only selective markers that are already in routine use in standard cloning vectors; and (iii) contain no harmful sequences. Some vectors may include sequences derived from mammalian (CMV) or insect viruses for the purpose of achieving a specific fate upon subsequent transfection of eukaryotic cells. These are generally restricted to well-characterised sequences that are completely harmless in

				isolation from other viral sequences. The transformations will be performed in DH5α. Plasmids for eukaryotic cell transfection may influence multiple parameters, such as cell growth, by affecting the activities of various cell cycle proteins. In addition, changes in the level of other unknown genes is currently being studied in the lab. Some constructs contain different regions of the specific protein and these are used to look at changes in interaction with other known interacting proteins. The plasmids used for eukaryotic expression do contain regions of genes that are potentially oncogenic driven by viral promoters such as SV40. In the absence of wild-type virus or gene-products that provide the functions of the missing viral genes, the vector is not able to generate a productive infection. Therefore they are considered safe (level II) for transient transfections in this state.

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

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

Vector(s) *	Virus Used for Vector Construction	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
p-adenoX	adenovirus	Dr. Paul Yaswen Uof Cal Berkely	ZNF217	Increased Cell proliferation. Changes in gene transcription

Fastbac	baculovirus	Invitrogen	ZNF217, CBP, TDG	Growth arrest of insect cells and protein production.

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen O YES X NO
- E1A oncogene X YES O NO
- Known oncogenes X YES, please specify _____ZNF217 _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

Note: HEK293 cells express T antigen and E1A. HeLa cells are known to infected with human papilloma virus, which is causative in cancer. HeLa cells express E6 and E7 proteins of HPV. It was not possible to find a report of HPV being expressed by HeLa cells. ZNF217 is a putative oncogene.

4.5 Will virus be replication defective? O YES X NO

The adenovirus can be propogated in HEK293 cell line. The conditions under which this virus can be propogated are described on page 2

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

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

The containment levels are Level 2 for adenovirus. This will be maintained for all experiments.

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X 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? xYES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____*Mus musculus* (mouse)_____

6.3 AUS protocol # _____2009-052_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ XNO

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

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X 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 details:_____ O NO

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

9.0 Insects

9.1 Do you use insects? O YES X 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 Do you use insects that require a permit from the CFIA permit? O YES O NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? 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
If YES, Please attach the CFIA permit & 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_____ X NO
If no, please proceed to Section 12.0

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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____ _____

13.0 Containment Levels

13.1 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 2+ O 3

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

X YES, permit # if on-campus BIO –LRCC- 0005
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:2012-07-12

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

___N/A_____

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

-Wash the site of injury well under running water

-See Occupational Health and Safety staffed with Registered Nurses for help and to file an incident report

-After hours go to Emergency

15.0 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:_____ July 30, 2012

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

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Fwd: RE: Fwd: Re: Biohazard forms - Torchia
Date: Tue, 31 Jul 2012 13:21:33 -0400
From: Jamie Gibbings <Jamie.Gibbings@LHSC.ON.CA>
To:jstanle2@uwo.ca
CC: Julie Horne <julie.horne@LawsonResearch.Com>, Paul Coakwell <Paul.Coakwell@LawsonResearch.Com>, Sally Major <Sally.Major@LawsonResearch.Com>, Trisha Carter <Trisha.Carter@LawsonResearch.Com>, jtorchia@uwo.ca

Hi Jennifer,

Here is the signed form. Approved

The consultant is assume the original AUP addresses safety precaution for unpacking the cell lines that are shipped in liquid nitrogen such as, unpacked in a well ventilated area.

Thanks,

Jamie Gibbings, CRSP
Safety Coordinator
Occupational Health & Safety Services
London Health Sciences Centre
T. 519-685-8500 x77068
P. 15641

Subject: Biohazard forms
From: Joe Torchia <jtorchia@uwo.ca>
Date: 7/12/2012 2:05 PM
To: 'Jennifer Stanley' <jstanle2@uwo.ca>

Hi Jennifer:

I have attached a word file of my revised Biohazard forms as well as the rebuttal for the various reviewer comments provided. I have also attached some PDFs which were not included in the previosu version. Please include them in addition to those already in the application.

Joe Torchia PhD
Associate Professor and Principal Investigator
Dept. of Oncology and Biochemistry
London Regional Cancer Program and The University of Western Ontario
London, Ontario
Canada

Attachments:

additional PDFs.pdf	2.0 MB
Biohazards rebuttal.docx	16.7 KB
biosafety form orginal revised.doc	516 KB

Committee members

These are the comments which have been highlighted by the Biohazards committee at various times in the 12 months:

1. "There are inconsistent statements throughout this form. The Committee needs a statement from the researcher that the viruses that are used do not contain wild type p/CIP or ZNf217. (If they do this will affect the containment requirements per the Public Health Agency of Canada)
   If that is the case, then Section 4.0 needs to be filled out properly. If viruses contain mutations that are not oncogenic then it is okay.
   The protocol will not be approved until this issue is clarified.
   The clarification should come from the PI rather than the technician."

   I have modified the application to clarify the specific application of the ZNF217 adenovirus (page 2). Note that the p/CIP project is no longer being pursued and consequently we do not work with the p/CIP virus. In addition, the description of the p/CIP project has been removed and has been replaced by a newly funded project.

   I have also included baculovirus as a biological agent which we routinely use on page 2.

Specific comments were also provided by Dr. Steve Barr

1. "In your summary of the research proposed, you have stated that CIP and ZNF217 are potential oncogenes, however in section 4.2 of your BARF, you state "the constructs generated and used in our laboratory are not oncogenic."

   This has been clarified in section 4.2. and on page 2 of the application

2. In section 4.6, it should be checked "yes" for "Will virus be infectious to humans or animals" since Adenoviruses are infectious. In Table 1.2, Adenovirus is listed , however it was not checked as to whether it is a human pathogen, animal pathogen or zoonotic.

   Section 4.6 has been corrected and clarified

3. Table 4.3, "Will genetic modifications involving viral vectors be made?", was checked no. This is okay as long as you are not using Adenoviruses to transduce genes. In our correspondence with Majdina, we were confused by the statement that "Adenovirus does not have the capability to infect regular human cells," which is incorrect.

   Regarding the uses of the ZNF217 adenovirus has been explained and expanded on page 2. In addition I have included a copy of the MSDS sheets for infectious substances regarding the uses and handling of adenoviruses.

4. "In Section 4.4, the use of SV40 large T antigen was checked "no", however this should be checked "yes" since your lab uses 293T cells, which contain SV40 large T antigen".

   This has been corrected and Section 4.4 is now checked Yes

   Other modifications:

   Plasmid maps for fastbac, adeno X have been included which were omitted in the previous application. In addition information for DH10bac cells, SF9 cell and SF21 cells has been provided as PDFs

New pdf info

Public Health Agency of Canada | Agence de la santé publique du Canada

Adenovirus types 1, 2, 3, 4, 5 and 7 - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Adenovirus types 1, 2, 3, 4, 5 and 7***

SYNONYM OR CROSS REFERENCE: ARD, acute respiratory disease, pharyngoconjunctival fever

CHARACTERISTICS: *Adenoviridae*; non-enveloped, icosahedral virions, 70-90 nm diameter, doubled-stranded, linear DNA genome.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, tonsillitis, cough and conjunctivitis; common cause of nonstreptococcal exudative pharyngitis among children under 3 years; more severe diseases include laryngitis, croup, bronchiolitis, or severe pneumonia; a syndrome of pharyngitis and conjunctivitis (pharyngoconjunctival fever) is associated with adenovirus infection

EPIDEMIOLOGY: Worldwide; seasonal in temperate regions, with highest incidences in the fall, winter and early spring; in tropical areas, infections are common in the wet and colder weather; annual incidence is particularly high in children; adenovirus types 4 and 7 are common among military recruits (ARD)

HOST RANGE: Humans

INFECTIOUS DOSE: >150 plaque forming units when given intranasally

MODE OF TRANSMISSION: Directly by oral contact and droplet spread; indirectly by handkerchiefs, eating utensils and other articles freshly soiled with respiratory discharge of an infected person; outbreaks have been related to swimming pools; possible spread through the fecal-oral route

INCUBATION PERIOD: From 1-10 days

COMMUNICABILITY: Shortly prior to and for the duration of the active disease

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available; cidofovir has shown promise in the treatment of adenoviral ocular infections.

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulfate

PHYSICAL INACTIVATION: Sensitive to heat>56°C; unusually stable to chemical or physical agents and adverse pH conditions

SURVIVAL OUTSIDE HOST: Resistance to chemical and physical agents allows for prolonged survival outside of the body. Adenovirus type 3 survived up to 10 days on paper under ambient conditions; adenovirus type 2 survived from 3-8 weeks on environmental surfaces at room temperature

SECTION V - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis

FIRST AID/TREATMENT: Mainly supportive therapy

IMMUNIZATION: Vaccine available for adenovirus types 4 and 7 (used for military recruits)

PROPHYLAXIS: None available

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: Ten cases documented up to 1988

SOURCES/SPECIMENS: Respiratory secretions

PRIMARY HAZARDS: Ingestion; droplet exposure of the mucous membrane

SPECIAL HAZARDS: Contact with feces from infected animals

SECTION VII - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues

PROTECTIVE CLOTHING: Laboratory coat; gloves when skin contact with infectious materials is unavoidable

OTHER PRECAUTIONS: None

SECTION VIII - HANDLING INFORMATION

SPILLS: Allow aerosols to settle; wearing protective clothing gently cover the spill with absorbent paper towel and apply 1% sodium hypochlorite starting at the perimeter and working towards the centre; allow sufficient contact time (30 min) before clean up

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization, incineration, chemical disinfection

STORAGE: In sealed containers that are appropriately labelled

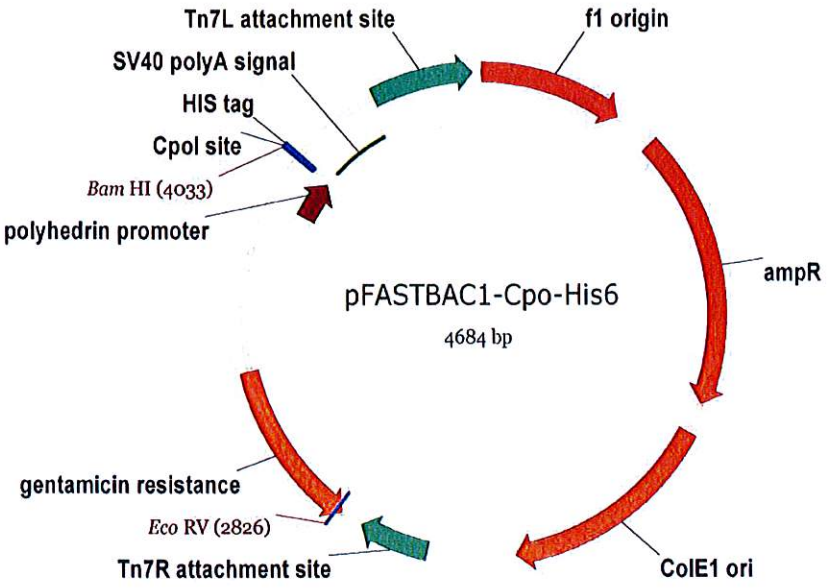
SECTION IX - MISCELLANEOUS INFORMATION

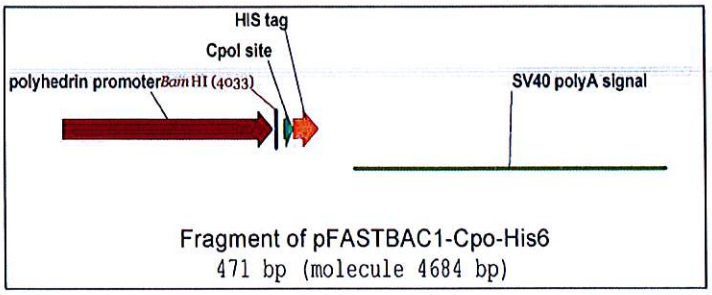
Date prepared: November 1999

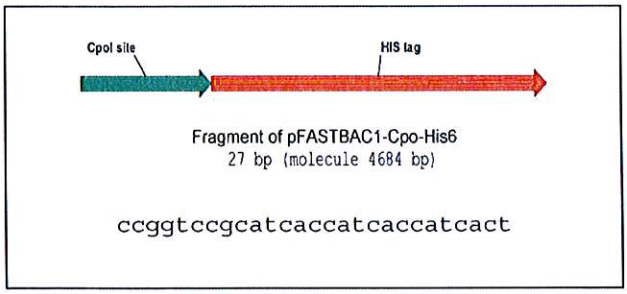
Prepared by: Office of Laboratory Security, PHAC

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or reliability or for any loss or injury resulting from the use of the information. Newly discovered hazards are frequent and this information may not be completely up to date.

Date Modified: 2001-01-23







Clone map from donor sequence
Some features annotated with A Plasmid Editor (M. Wayne Davis)
Map generated with VectorNTI (Invitrogen)
Courtesy of DF/HCC DNA Resource Core's Plasmid Information Database (PlasmID)
http://dnaseq.med.harvard.edu
http://plasmid.hms.harvard.edu

GIBCO™
Invitrogen Corporation

Sf21 Cells

Cat. No. 11497-013

Shipping and Storage

Cells are supplied in a cryogenic vial containing 1.5 x 10^7 viable cells in a volume of 1.5 mL. **Store in liquid nitrogen (vapor phase).**

Caution

This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

General Media Requirements

Suspension or adherent culture: Use Sf-900 II SFM as is. Sf-900 II SFM contains L-Glutamine and does not require additional supplements. Protect medium from light.

Note: Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin may be used when required.

Thawing Cells

Store frozen Sf21 cells in liquid nitrogen (vapor phase) until ready to use. Frozen cells are supplied in and may be thawed directly into Sf-900 II SFM. Use the following procedure to thaw cells.

Note: We recommend thawing Sf21 cells into suspension culture in shake flasks. Do not thaw Sf21 cells directly into spinner vessels. For spinner culture applications, thaw cells into shake flasks using the procedure below. Carry and expand cells in shake flasks for 2 to 3 passages, then seed cells into spinner vessels.

1. Rapidly thaw frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a 125 mL shake flask containing 27 mL of pre-warmed Sf-900 II SFM, and incubate in a 28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm. Loosen caps of flasks to allow oxygenation/aeration.
2. Once the culture density has reached >2 x 10^6 viable cells/mL, determine viable and total cell counts (see procedure).
3. Expand Sf21 cultures by seeding shake flasks at 3 to 5 x 10^5 viable cells/mL by diluting cells in pre-warmed growth medium. See **Subculturing Cells** to maintain and subculture Sf21 cells in suspension or adherent culture.

Note: We recommend subculturing cells for a minimum of 3 passages before use in other applications.

Determining Cell Density and Viability

Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability using the trypan blue exclusion method.
3. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

Subculturing Cells

Use the recommended conditions, procedure, and tips to subculture Sf21 cells. We recommend thawing a fresh, low-passage culture of frozen Sf21 cells every 3 months or 30 passages. For more information about maintaining suspension and adherent cultures, refer to the *Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques*, which is available for downloading from our Web site (www.invitrogen.com).

Recommended Conditions

	Suspension Cultures	Adherent Cultures
Cell density	>2 x 10^6 viable cells/mL	>80% confluent
Culture vessel	125 or 250 mL disposable, sterile Erlenmeyer flask containing 35-50 mL or 75-100 mL total working volume of cell suspension, respectively **Note:** Glass flasks without baffles may be used, but be sure to clean flasks thoroughly after each use to avoid potential toxicity.	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 mL for T-75 cm^2 flasks and 40-50 mL for T-162 cm^2 flasks
Seeding density	3 to 5 x 10^5 viable cells/mL	2 to 5 x 10^4 viable cells/cm^2
Incubation conditions	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm; loosen caps to allow for oxygenation/aeration	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room; loosen caps to allow for oxygenation/ aeration

Subculturing Procedure

1. **Suspension cultures:** Proceed to Step 2. **Adherent cultures:** Remove medium and floating cells from a confluent monolayer and discard. Add 10 mL of fresh growth medium to a T-75 cm^2 flask (20 mL to a T-162 cm^2 flask). Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask). Transfer the cell suspension into a centrifuge tube.
2. Determine viable and total cells counts (see procedure).
3. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

Subculturing Tips

Suspension cultures: To reduce accumulation of cell debris and metabolic waste by-products in shaker cultures, we recommend gently centrifuging the cell suspension at 100 x g for 5 to 10 minutes and resuspending the cell pellet in fresh Sf-900 II SFM once every 3 weeks.

Adherent cultures: For slower growing adherent cultures, we suggest removing spent media and replacing with fresh growth media every 3 to 4 days until Sf21 cells are ready to subculture.

Scaling-Up Sf21 Cells into Spinner Culture

You may scale-up the Sf21 cultures in spinner flasks using the guidelines below. Note that the appropriate spinner or impeller speed and seeding density should be determined and optimized for each system. For spinners >500 mL, use a vessel that provides for gas sparging.

- **Spinner culture volume:** The total culture volume should not exceed 60% of the indicated volume of spinner for proper aeration (*e.g.* a 250 mL spinner should not contain >150 mL of culture).
- **Spinner or impeller speed:** Determine the optimum impeller speed for your spinner vessel depending on your needs. To reduce loss of viability due to cell shearing, make sure that the impeller blade rotates freely and does not contact vessel walls or base.
- **Seeding density:** We use optimized seeding densities of 3 to 5 x 10^5 viable cells/mL and subculture cells when they reach a density of >2 x 10^6 viable cells/mL.

Freezing Cells

Recommended Conditions

- Freeze cells at a density of ≥1 x 10^7 viable cells/mL.
- Use a freezing medium composed of 50% fresh growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned media collected from Sf21 cultures during subculture procedure) and DMSO to a final concentration of 7.5%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

Freezing Procedure

1. Grow the desired quantity of Sf21 cells in shake or spinner flasks, harvesting when the cells are in mid-log exponential growth and have a viability of >90%.
2. Determine viable and total cell counts (see procedure on the previous page) and calculate the volume of freezing medium required to yield a final cell density of ≥1x10^7 viable cells/mL.
3. Prepare the required volume of freezing medium (see above).
4. Centrifuge cells from cell suspension (Step 1) at 100 x g for 5 to 10 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications (*i.e.* 1.5 mL in a 2 mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
7. Transfer frozen vials to liquid nitrogen (vapor phase) storage.

Note: You may check the viability and recovery of frozen cells 24 hours after storing vials in liquid nitrogen by following the procedure outlined in **Thawing Cells**.

Transfection

For optimal results, we recommend using Cellfectin® Reagent available from Invitrogen for transfection. Refer to the manual accompanying the product for instructions. Note that if you use Cellfectin® Reagent, you may transfect cells directly in Sf-900 II SFM. Other transfection reagents are suitable.

General Information

The Sf21 insect cell line is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE[1,2], and is a suitable host for expression of recombinant proteins from baculovirus expression systems[3,4] (*e.g.* Invitrogen's Bac-to-Bac® and Bac-N-Blue™ Expression Systems).

The Sf21 cell line exhibits the following general features:

- Prepared from low passage Master Cell Bank cultures that are only 100 to 110 total passages and 10 to 20 passages serum-free.
- Adapted to serum-free suspension growth in Sf-900 II SFM, a serum-free medium optimized for growth of Sf21 and other invertebrate cell lines[5]. **Note:** Cells also grow well in traditional media supplemented with serum[6] (*i.e.* Grace's Supplemented (TNM-FH) Insect Cell Culture Medium supplemented with 10% heat-inactivated fetal bovine serum).

Product Qualification

Frozen catalog Sf21 cells are performance tested for viability and cell growth post-recovery from cryopreservation, and are screened for mycoplasma and sterility. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Species identity is confirmed by isozyme and karyotype analysis.

References

1. Gardiner, G.R. and Stockdale, H. (1975) *J. Invertebr. Pathol. 25*, 363.
2. Vaughn, J.L., Goodwin, R.H., Tompkins, G.J., and McCawley, P. (1977) *In Vitro 13*, 213.
3. Smith, G.E., Summers, M.D. and Fraser, M.J. (1983) *Mol. Cell. Biol. 3*, 2156.
4. Luckow, V.A. and Summers, M.D. (1988) *Bio/Technology 6*, 47.
5. Godwin, G. and Whitford, W. (1993) *Focus 15*, 44.
6. Grace, T.D.C. (1962) *Nature 195*, 788.

Related Products

	Quantity	Cat. No.
Sf-900 II SFM	1 L	10902-088
Penicillin-Streptomycin	100 mL	15070-063
Grace's Insect Cell Culture Medium, Supplemented	500 mL	11605-094
Fetal Bovine Serum, Heat-Inactivated	500 mL	10082-147
Cellfectin® Reagent	1 mL	10362-010

Note: Other reagent sizes are available.

For further information on this or other GIBCO™ products, contact Technical Services at the following:

United States TECH-LINE℠: 1 800 955 6288
Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

December 2002 **Form No. 3911**

Subject: Re: Inspection follow-up
From: dmbryce@uwo.ca
Date: 7/29/2012 1:41 PM
To: Jennifer Stanley <jstanle2@uwo.ca>

Hello Jennifer,
I have completed all of the recommendations pursuant to the inspection of Dr. Beier laboratory. The TDG training had been the only outstanding item, and that is now complete. I have submitted the quizzes for both Biological and Dry Ice, and Category A Only courses. I am not sure whether they will respond to me directly with my evaluation, or if the results will be sent to you. My access to the course was time-limited and will expire on July 31st. The format of the individual pages of the course was such that I did not print out copies. (It would not have been environmentally conscious) I would however like to request an updated booklet of the TDG regulations to maintain in the lab for future reference. Would you have any copies available?
Thank you, and I hope you are having a wonderful summer,
Dawn Bryce
Sent from my BlackBerry device on the Rogers Wireless Network

---

From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 17 Jul 2012 19:26:05 -0400
To: Frank Beier<fbeier@uwo.ca>
Cc: <dmbryce@uwo.ca>
Subject: Re: Inspection Results: Beier lab - DSB 0033

Hi there
Please confirm that these items have now been addressed.
Regards,
Jennifer

On 6/13/2012 1:20 PM, Jennifer Stanley wrote:

> Dawn
> You should get information by email from lsd-dsl@phac-aspc.gc.ca
> jennifer
>
> On 6/12/2012 12:54 PM, Dawn-Marie Bryce wrote:
>
> > Hi Jennifer,
> > I logged on to my WebCT and there is no Transportation of Dangerous Goods Course listed for me.
> > Would you be able to adjust the course listing so that I may do the online course?
> > Thanks,
> > Dawn

On 06/11/12, **Jennifer Stanley** <jstanle2@uwo.ca> wrote:

Thanks
Let me know when you have completed the training (tomorrow)
Jennifer

On 6/11/2012 2:25 PM, Dawn-Marie Bryce wrote:

Hi Jennifer,
The signs have been modified to list existing Level 2 hazards, hand sanitizer has been placed in the tissue culture area and hooks have been installed for tissue culture dedicated lab jackets. I will log on and complete the Transportation of Dangerous Goods update training tomorrow.
Thanks,
Dawn

On 06/05/12, **Jennifer Stanley** <jstanle2@uwo.ca> wrote:

See location change

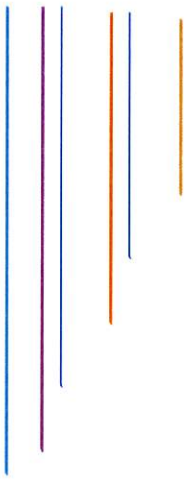
On 6/5/2012 2:20 PM, Jennifer Stanley wrote:

DSB 0033 was inspected on 5/1/2012. Several things were done well, including:
There are good training records.
The biohazard waste containers are well labelled.
The lab is kept very clean and tidy.

There are a few items that need to be addressed:
- The Level 2 signs need to have the Level 2 hazards listed.
- Sanitizer needs to be used before exiting the room (no hand sinks available).
- Dawn's dangerous goods training has expired and needs to be re-freshed. She has been signed up for a free, on-line course.

Please resolve these issues and reply back by email no later than June 15, 2012

Regards
Jennifer



GIBCO™
Invitrogen Corporation

Sf9 Cells

Cat. No. 11496-015

Shipping and Storage

Cells are supplied in a cryogenic vial containing 1.5 x 10^7 viable cells in a volume of 1.5 mL. **Store in liquid nitrogen (vapor phase).**

Caution

This product contains Dimethyl Sulfoxide (DMSO), a hazardous material. Review the Material Safety Data Sheet before handling.

General Media Requirements

Suspension or adherent culture: Use Sf-900 II SFM as is. Sf-900 II SFM contains L-Glutamine and does not require additional supplements. Protect media from light.

Note: Antibiotics are not recommended; however, 5 mL/L of Penicillin-Streptomycin may be used when required.

Thawing Cells

Store frozen Sf9 cells in liquid nitrogen (vapor phase) until ready to use. Frozen cells are supplied in and may be thawed directly into Sf-900 II SFM. Use the following procedure to thaw cells.

Note: We recommend thawing Sf9 cells into suspension culture in shake flasks. Do not thaw Sf9 cells directly into spinner vessels. For spinner culture applications, thaw cells into shake flasks using the procedure below. Carry and expand cells in shake flasks for 2 to 3 passages, then seed cells into spinner vessels.

1. Rapidly thaw frozen vial in a 37°C water bath. Triturate and transfer the entire contents of the cryovial into a 125 mL shake flask containing 27 mL of pre-warmed Sf-900 II SFM, and incubate in a 28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm. Loosen caps of flasks to allow oxygenation/aeration.
2. Once the culture density has reached >2 x 10^6 viable cells/mL, determine viable and total cell counts (see procedure).
3. Expand Sf9 cultures by seeding shake flasks at 3 to 5 x 10^5 viable cells/mL by diluting cells in pre-warmed growth medium. See **Subculturing Cells** to maintain and subculture Sf9 cells in suspension or adherent culture.

Note: We recommend subculturing cells for a minimum of 3 passages before use in other applications.

Determining Cell Density and Viability

Follow the procedure below to determine viable and total cell counts.

1. Transfer a small aliquot of the cell suspension to a microcentrifuge tube.
2. Determine viability using the trypan blue exclusion method.
3. Determine cell density electronically using a Coulter Counter or manually using a hemocytometer chamber.

Subculturing Cells

Use the recommended conditions, procedure, and tips to subculture Sf9 cells. We recommend thawing a fresh, low-passage culture of frozen Sf9 cells every 3 months or 30 passages. For more information about maintaining suspension and adherent cultures, refer to the *Guide to Baculovirus Expression Vector Systems (BEVS) and Insect Cell Culture Techniques*, which is available for downloading from our Web site (www.invitrogen.com).

Recommended Conditions

	Suspension Cultures	Adherent Cultures
Cell density	>2 x 10^6 viable cells/mL	>80% confluent
Culture vessel	125 or 250 mL disposable, sterile Erlenmeyer flask containing 35-50 mL or 75-100 mL total working volume of cell suspension, respectively **Note:** Glass flasks without baffles may be used, but be sure to clean flasks thoroughly after each use to avoid potential toxicity.	T-75 cm^2 to T-162 cm^2 disposable sterile T-flasks. Dilute cells in a total working volume of 15-20 mL for T-75 cm^2 flasks and 40-50 mL for T-162 cm^2 flasks
Seeding density	3 to 5 x 10^5 viable cells/mL	2 to 5 x 10^4 viable cells/cm^2
Incubation conditions	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room on an orbital shaker platform rotating at 125-150 rpm; loosen caps to allow for oxygenation/aeration	28°C ± 0.5°C non-humidified, ambient air-regulated incubator or warm room; loosen caps to allow for oxygenation/ aeration

Subculturing Procedure

1. **Suspension cultures:** Proceed to Step 2. **Adherent cultures:** Remove medium and floating cells from a confluent monolayer and discard. Add 10 mL of fresh growth medium to a T-75 cm^2 flask (20 mL to a T-162 cm^2 flask). Displace cells from the flask's surface by rapping the flask sharply against your hand 3 or 4 times (>75% of the cells should be detached from the surface of the flask). Transfer the cell suspension into a centrifuge tube.
2. Determine viable and total cells counts (see procedure).
3. Seed cells at the recommended density (see table), diluting in pre-warmed growth medium. Put flasks in incubator with caps loosened to allow for oxygenation/aeration.

Subculturing Tips

Suspension cultures: To reduce accumulation of cell debris and metabolic waste by-products in shaker cultures, we recommend gently centrifuging the cell suspension at 100 x g for 5 to 10 minutes and resuspending the cell pellet in fresh Sf-900 II SFM once every 3 weeks.

Adherent cultures: For slower growing adherent cultures, we suggest removing spent media and replacing with fresh growth media every 3 to 4 days until Sf9 cells are ready to subculture.

Scaling-Up Sf9 Cells into Spinner Culture

You may scale-up the Sf9 cultures in spinner flasks using the guidelines below. Note that the appropriate spinner or impeller speed and seeding density should be determined and optimized for each system. For spinners >500 mL, use a vessel that provides for gas sparging.

- **Spinner culture volume:** The total culture volume should not exceed 60% of the indicated volume of spinner for proper aeration (*e.g.* a 250 mL spinner should not contain >150 mL of culture).
- **Spinner or impeller speed:** Determine the optimum impeller speed for your spinner vessel depending on your needs. To reduce loss of viability due to cell shearing, make sure that the impeller blade rotates freely and does not contact vessel walls or base.
- **Seeding density:** We use optimized seeding densities of 3 to 5 x 10^5 viable cells/mL and subculture cells when they reach a density of >2 x 10^6 viable cells/mL.

Freezing Cells

Recommended Conditions

- Freeze cells at a density of ≥1 x 10^7 viable cells/mL.
- Use a freezing medium composed of 50% fresh growth medium and 50% conditioned growth medium (day 2 to 4 cell conditioned media collected from Sf9 cultures during subculture procedure) and DMSO to a final concentration of 7.5%. Prepare freezing medium immediately before use. Filter-sterilize the freezing medium and chill at 4°C until use. Discard any remaining freezing medium after use.

Freezing Procedure

1. Grow the desired quantity of Sf9 cells in shake or spinner flasks, harvesting when the cells are in mid-log exponential growth and have a viability of >90%.
2. Determine viable and total cell counts (see procedure on the previous page) and calculate the volume of freezing medium required to yield a final cell density of ≥1x10^7 viable cells/mL.
3. Prepare the required volume of freezing medium (see above).
4. Centrifuge cells from cell suspension (Step 1) at 100 x g for 5 to 10 minutes. Aseptically decant supernatant and resuspend the cell pellet in the pre-determined volume of chilled freezing medium.
5. Dispense aliquots of this suspension (frequently mixing to maintain a homogeneous cell suspension) into cryovials according to manufacturer's specifications (*i.e.* 1.5 mL in a 2 mL cryovial).
6. Freeze cells in an automated, controlled-rate freezing apparatus or using a manual method following standard procedures. For ideal cryopreservation, the freezing rate should be a decrease of 1°C per minute.
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Transfection

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General Information

The Sf9 insect cell line is a clonal isolate derived from the parental *Spodoptera frugiperda* cell line IPLB-Sf-21-AE[1,2], and is a suitable host for expression of recombinant proteins from baculovirus expression systems[3,4] (*e.g.* Invitrogen's Bac-to-Bac® and Bac-N-Blue™ Expression Systems).

The Sf9 cell line exhibits the following general features:

- Prepared from low passage Master Cell Bank cultures that are only 40 to 50 total passages and 10 to 20 passages serum-free.
- Adapted to serum-free suspension growth in Sf-900 II SFM, a serum-free medium optimized for growth of Sf9 and other invertebrate cell lines[5]. **Note:** Cells also grow well in traditional media supplemented with serum[6] (*i.e.* Grace's Supplemented (TNM-FH) Insect Cell Culture Medium supplemented with 10% heat-inactivated fetal bovine serum).

Product Qualification

Frozen catalog Sf9 cells are performance tested for viability and cell growth post-recovery from cryopreservation, and are screened for mycoplasma and sterility. Master Cell Banks are screened for viruses, mycoplasma, and sterility. Species identity is confirmed by isozyme and karyotype analysis.

References

1. Smith, G.E., Ju, G., Ericson, B.L., Moshera, J., Lahm, H., Chizzonite, R. and Summers, M.D. (1985) *Proc. Natl. Acad. Sci. USA 82*, 8404.
2. Vaughn, J.L., Goodwin, R.H., Tompkins, G.J., and McCawley, P. (1977) *In Vitro 13*, 213.
3. Smith, G.E., Summers, M.D. and Fraser, M.J. (1983) *Mol. Cell. Biol. 3*, 2156.
4. Luckow, V.A. and Summers, M.D. (1988) *Bio/Technology 6*, 47.
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Related Products

	Quantity	Cat. No.
Sf-900 II SFM	1 L	10902-088
Penicillin-Streptomycin	100 mL	15070-063
Grace's Insect Cell Culture Medium, Supplemented	500 mL	11605-094
Fetal Bovine Serum, Heat-Inactivated	500 mL	10082-147
Cellfectin® Reagent	1 mL	10362-010

Note: Other reagent sizes are available.

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Canada TECH-LINE: 1 800 757 8257

Outside the U.S. and Canada, refer to the GIBCO products catalogue for the TECH-LINE in your region.

You may also contact your Invitrogen Sales Representative or our World Wide Web site at www.invitrogen.com.

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For research use only.
CAUTION: Not intended for human or animal diagnostic or therapeutic uses.

December 2002 **Form No. 3910**

SUMMARY OF THE ANIMAL WORK

Under Animal Use Protocol # 2009-052 (AUS #2009-052) titled "Functional analysis of transcriptional coregulators in the mouse" we currently have a mouse model overexpressing ZNF217 in a tissue specific manner. We are currently expanding the line by breeding the animals containing a single copy of the transgene to *wild-type* animals. Resulting offspring are tagged and tailed at 14 days postpartum, and weaned at 21 days postpartum. Only animals containing the transgene are kept while the *wild-type* mice are sacrificed as explained in the AUS. Line of experimentation involves sacrificing the animal (as described in the AUS) and tissue collection for analysis of transgene expression and its effects on tissues expressed in. All mouse carcasses and tissues are disposed in accordance to the AUS# 2009-052.

BL21

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Bacteria

ATCC® Number: BAA-1025™ Order this Item

Price: $205.00

Organism: *Escherichia coli* (Migula) Castellani and Chalmers

Designations: BL21

Depositor: J Bull

History: J Bull I J Molineux

Biosafety Level: 1

Shipped: freeze-dried

Growth Conditions: ATCC medium129: Nutrient agar with 0.5% NaCl
Temperature: 37.0°C
Duration: aerobic

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.

Applications: bacteriophage host (host for bacteriophages T3 and T7)

Related Products: bacteriophage:ATCC BAA-1025-B1
bacteriophage:ATCC BAA-1025-B2

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DH5α

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ATCC® Number:	68233™ Order this Item	**Price:**	$200.00
Designation / Description:	Escherichia coli DH5 alpha, pDSRG, SCC 2197		
U.S. Patent Number:	5,599,906		
Biosafety Level:	1		
Shipped:	room temperature		
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.		

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Patent Depository

ATCC® Number:	PTA-3100™ Order this Item	**Price:**	$200.00
Designation / Description:	Insect Cell line, Sf9-P35AcV5-3		
U.S. Patent Number:	7,405,038		
Biosafety Level:	1		
Shipped:	frozen		
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.		

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mES-R1

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Cell Biology

ATCC® Number: SCRC-1011™ Order this Item **Price:** $649.00

Designations:	R1
Depositors:	A Nagy
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	spherical colony
Source:	**Strain:** 129X1 x 129S1 **Organ:** embryo **Tissue:** inner cell mass **Cell Type:** embryonic stem cell;
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.
Restrictions:	Prior to purchase, for-profit commercial institutions must obtain a license agreement. For instructions on how to proceed, please contact ATCC's Office of Licensing and Business Development at licensing@ATCC.org or 703 365 2773.
Isolation:	**Isolation date:** August, 1991
Age:	3.5 days embryo, blastocyst
Gender:	male
Comments:	The R1 cell line was established in August 1991, from a 3.5 day blastocyst produced by crossing two 129 substrains (129S1/SvImJ and 129X1/SvJ). The cells are heterozygous for the c locus (+/c (ch)) and for the pink eye locus (+/p). In the F1 generation the coat color is uniform agouti, while in the F2 these two coat color genes segregate. The segregation could result in several coat types, from albino, through light brown, to black, depending on the genetic background of the partner of the germline chimaera. Pluripotency of R1 was initially tested by tetraploid embryo <-> ES aggregates for completely ES derived development [PubMed: 8378314]. They were also tested by diploid embryo <-> ES aggregates and blastocyst injection for germline transmission in chimeras [PubMed: 8361547]. At early passages (up to passage #14), one third of the completely R1-derived newborns generated by tetraploid embryo <-> R1 aggregates survived. No live offspring were produced from cells older than passage #14.* . However, about 20% of subclones derived from passage #14 had the original developmental potential of R1 when tested by tetraploid aggregates [PubMed: 8378314]]. R1-derived animals reached adulthood and were fertile. The genetically altered lines derived from R1 gave high efficiency of germline transmission either by injecting them into C57 blastocyst or aggregating them with CD-1 or ICR outbred 8-cell stage embryos. More than 90% of the individual K.O. clones went to germline (n>60) by aggregation chimeras. **Current ATCCstocks of R1 cells are beyond passage 14. Current stocks of alternative subclone of R1 cells, designated R1/E (ATCCSCRC-1036), are below passage 14 and have been shown to be germline competent.*
Propagation:	ATCC complete growth medium: ES-DMEM (ATCC SCRR-2010) supplemented with 2.0 mM L-Alanyl-L-Glutamine (ATCC 30-2115), 0.1 mM non-essential Amino Acids (ATCC 30-2116), 0.1 mM 2-mercaptoethanol (Invitrogen Life Technologies No. 21985), 1000 U/ml mouse leukemia inhibitory factor (LIF) (Chemicon No. ESG1107) and 15% fetal bovine serum (ATCC SCRR-30-2020). **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Establishing and maintaining your culture:To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells. 1. Plate mitotically arrested MEF (CF-1) (ATCCSCRC-1040) as a feeder layer at approximately 1.5 to 2.0 X 10(6) cells/T25 at least one day before plating R1 cells (see product sheet for mitotically arrested MEF for protocol). One hour before thawing the vial of R1 ES cells, perform a 100% medium change using

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HT-1080

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Cell Biology

ATCC® Number: CCL-121™ Order this Item **Price:** $272.00

Designations:	HT-1080
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Tissue:** connective tissue **Disease:** fibrosarcoma
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.
Isolation:	**Isolation date:** July, 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Human poliovirus 1 RD-114 Feline Feline leukemia virus Vesicular stomatitis virus
Tumorigenic:	Yes
Oncogene:	ras +
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 12,14 D16S539: 9,12 D5S818: 11,13 D7S820: 9,10 THO1: 6 TPOX: 8 vWA: 14,19
Cytogenetic Analysis:	modal number = 46; range = 44 to 48. Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.
Isoenzymes:	G6PD, B
Age:	35 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells contain an activated N-ras oncogene.
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%. **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.

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293T

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Cell Biology

ATCC® Number: CRL-11268™ Order this Item Price: $272.00

Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	Organ: kidney
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.
Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCCby Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
Age:	fetus
Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCCCRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCCCRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCCCRL-11554) amphotropic envelope-expression packaging cell line.
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%. **Temperature:** 37.0°C **Atmosphere:** air, 95%, carbon dioxide (CO2), 5%
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.

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Cell Biology

ATCC® Number: CCL-2™ Order this Item **Price:** $256.00

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 (Cells contain human papilloma virus)
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
	
Source:	Organ: cervix Disease: adenocercinoma 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 [26623]
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.
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
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

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Cell Biology

ATCC® Number: HTB-22™ Order this Item **Price:** $272.00

Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
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.
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: 8,8 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%

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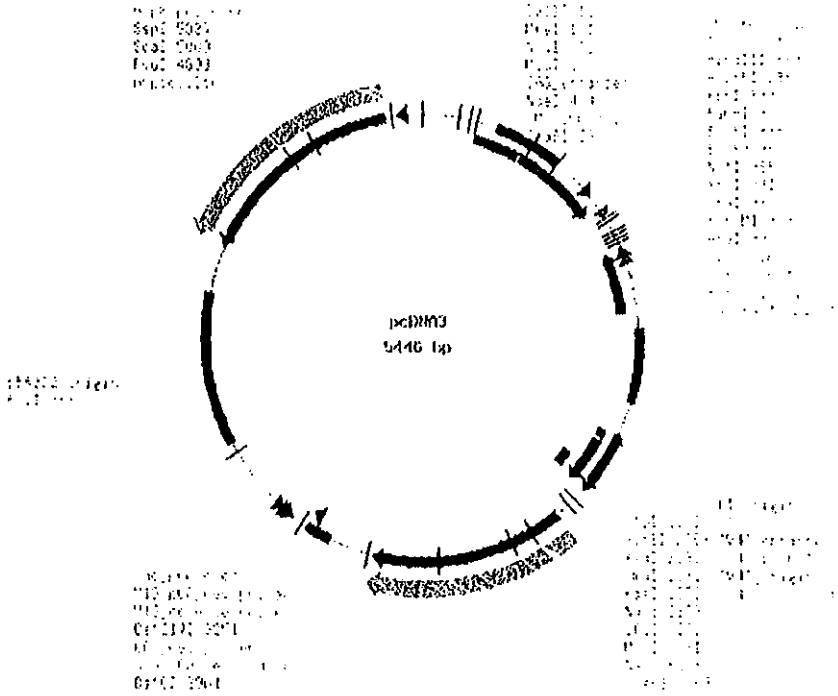
Plasmid Name	pcDNA3
Source/Vendor	Invitrogen
Plasmid Type	Mammalian expression
Promoter	CMV
Plasmid Size	5446
Sequencing Primer	T7
Bacterial Resistance	Ampicillin
Mammalian Selection	Neomycin
Plasmid Sequence	View Sequence


pcDNA3
5446 bp

invitrogen
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pFastBac Dual
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Vector Name	Map	Polylinker	Sequence	Restriction
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pFastBac Dual	[GIF]	[GIF]	[GIF]	[GIF]

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Cat. No.	4318709	Cat. No.	11668-019	Cat. No.	31053-028
Unit Size	1 kit	Unit Size	1.5 ml	Unit Size	500 ml
Price(CAD)	362.00	Price(CAD)	610.00	Price(CAD)	39.25
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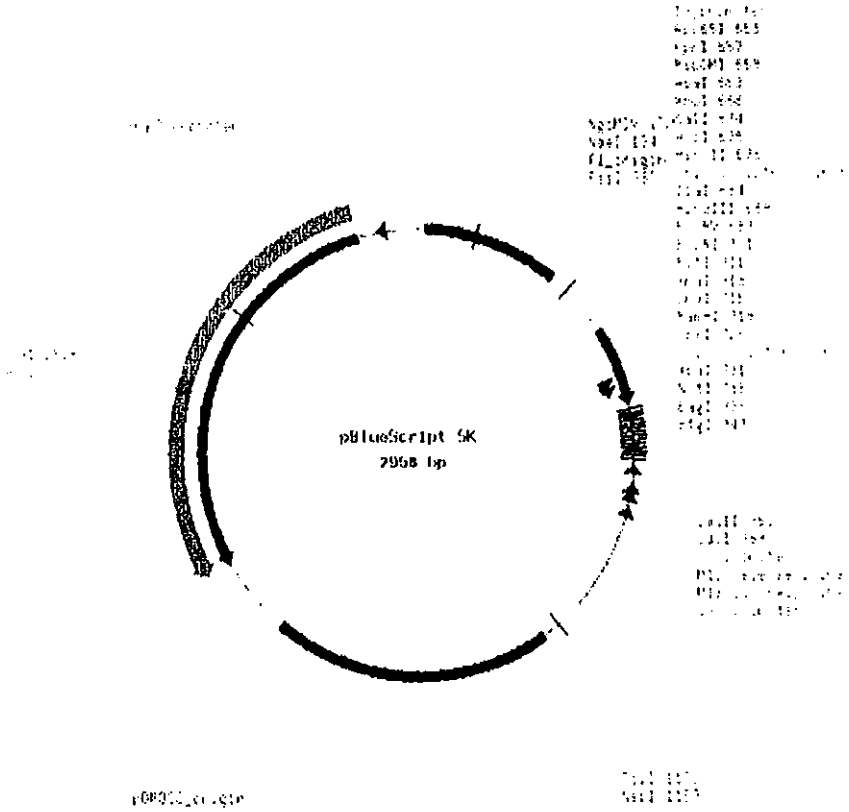
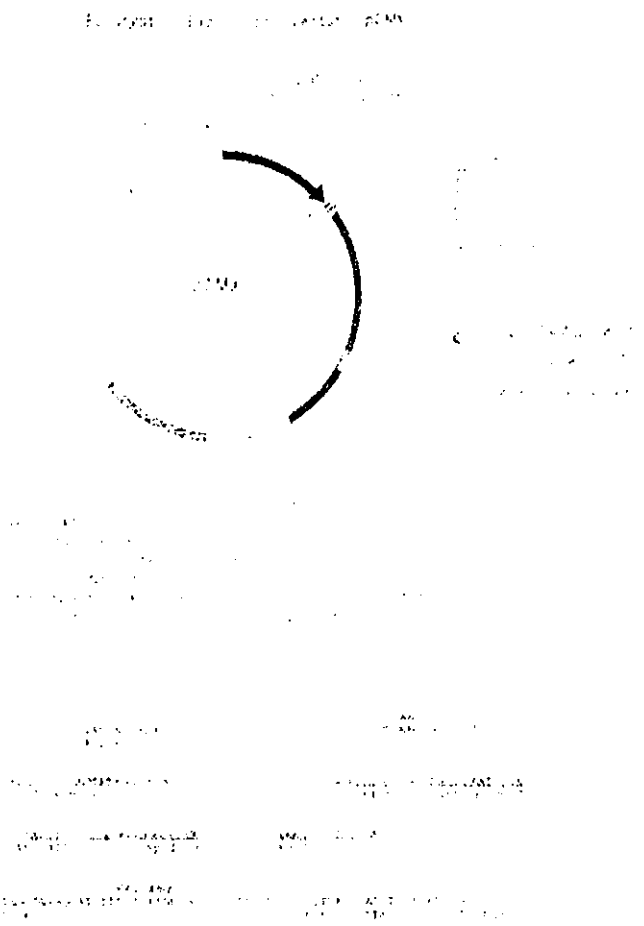

pBlueScript SK
2958 bp

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pCMX
Plasmid Type	Mammalian
Viral/Non-viral	non-viral
Constitutive/inducible	constitutive
Promoter	CMV
Plasmid Size	4500
Bacterial Resistance	Ampicillin
Notes	Please see K. Umesono et al., (1991) Cell 65: 1255-1266. The map shown is from Inder Vern lab. The wild-type version of pCMX plasmid does not contain IkB.

Maps



pGEX Vectors (GST Gene Fusion System)

- **A tac promoter for chemically inducible, high-level expression of GST-tagged recombinant proteins.**
- An internal $lacI^q$ gene for use in any E. coli host.
- Very mild elution conditions for release of fusion proteins from the affinity matrix, thus minimizing effects on antigenicity and functional activity.
- PreScission™ Protease, Thrombin, or Factor Xa recognition sites for cleaving the desired protein from the fusion product.

Page down for more information

Order Information

Product	Pack size	Product Code	Price	Qty	
Glutathione S-transferase Gene Fusion Vectors					
pGEX-1λT EcoRI/BAP*	5 µg	28-9546-56	CAN$456.00		add + add to hotlist
pGEX-2T†	25 µg	27-4801-01	CAN$617.00		add + add to hotlist
pGEX-2T*	25 µg	28-9546-53	CAN$671.00		add + add to hotlist
pGEX-2TK†	25 µg	27-4587-01	CAN$678.00		add + add to hotlist
pGEX-3X†	25 µg	27-4803-01	CAN$635.00		add + add to hotlist
pGEX-4T-1†	25 µg	27-4580-01	CAN$617.00		add + add to hotlist
pGEX-4T-1*	25 µg	28-9545-49	CAN$671.00		add + add to hotlist
pGEX-4T-2†	25 µg	27-4581-01	CAN$617.00		add + add to hotlist
pGEX-4T-2*	25 µg	28-9545-50	CAN$671.00		add + add to hotlist
pGEX-4T-3†	25 µg	27-4583-01	CAN$659.00		add + add to hotlist
pGEX-4T-3*	25 µg	28-9545-52	CAN$671.00		add + add to hotlist
pGEX-5X-1†	25 µg	27-4584-01	CAN$659.00		add + add to hotlist
pGEX-5X-1*	25 µg	28-9545-53	CAN$671.00		add + add to hotlist

pGEX-5X-2[†]	25 µg	27-4585-01	CAN$678.00		add + add to hotlist
pGEX-5X-3[†]	25 µg	27-4586-01	CAN$678.00		add + add to hotlist
pGEX-6P-1[*]	25 µg	28-9546-48	CAN$671.00		add + add to hotlist
pGEX-6P-2[†]	25 µg	27-4598-01	CAN$617.00		add + add to hotlist
pGEX-6P-2[*]	25 µg	28-9546-50	CAN$671.00		add + add to hotlist
pGEX-6P-3[*]	25 µg	28-9546-51	CAN$671.00		add + add to hotlist

[*] E. coli *BL21 is not supplied with the vector. E. coli BL21 can be ordered separately, see E. coli BL21.*
[†] E. coli *BL21 included with vector.*

You may also need:

Product	Pack size	Product Code	Price	Qty	
2'-Deoxyadenosine 5'-Triphosphate, Disodium, Crystalline (dATP)	250 mg	27-1850-04	CAN$856.00		add + add to hotlist
DRigest III (λ DNA-*Hind* III/φX-174 RF DNA-*Hae* III Digest)	25 µg	27-4060-01	CAN$255.00		add + add to hotlist
2-D Clean-Up Kit	50 samples	80-6484-51	CAN$370.00		add + add to hotlist
Anti-GST Antibody	0.5 ml, 50 detections	27-4577-01	CAN$445.00		add + add to hotlist
Adenosine 5'-Triphosphate, Disodium, Crystalline (ATP)	25 g	27-1006-03	CAN$526.00		add + add to hotlist
M13KO7 Helper Phage	100 µl	27-1524-01	CAN$86.00		add + add to hotlist

pGEX Vectors (GST Gene Fusion System)

Technical Information

Map of the glutathione S-transferese fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

Thirteen pGEX vectors are available with or without *E. coli* BL21 (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 Thrombinsee Thrombin recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX-5X-3 are derivatives of pGEX-3X and possess a Factor Xa see Factor Xarecognition site.

Download the pGEX sequence map in PDF format. For ASCII format please scroll 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-P^{32}]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-1λT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from λ gt11 libraries.

Vector	Unformatted	Formatted	GenBank Accession No.
pGEX-1λT, 28-9184-41 AB	*ASCII*	*PDF*	U13849
pGEX-2T, 28-9184-42 AB	*ASCII*	*PDF*	U13850
pGEX-2TK, 28-9189-67 AB	*ASCII*	*PDF*	U13851
pGEX-3X, 28-9184-43 AB	*ASCII*	*PDF*	U13852
pGEX-4T-1, 28-9184-44 AB	*ASCII*	*PDF*	U13853
pGEX-4T-2, 28-9184-45 AB	*ASCII*	*PDF*	U13854
pGEX-4T-3, 28-9184-46 AB	*ASCII*	*PDF*	U13855
pGEX-5X-1, 28-9184-47 AB	*ASCII*	*PDF*	U13856
pGEX-5X-2, 28-9184-48 AB	*ASCII*	*PDF*	U13857
pGEX-5X-3, 28-9184-49 AB	*ASCII*	*PDF*	U13858
pGEX-6P-1, 28-9184-50 AB	*ASCII*	*PDF*	U78872
pGEX-6P-2, 28-9184-51 AB	*ASCII*	*PDF*	U78873
pGEX-6P-3, 28-9184-53 AB	*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 M_r 26 000 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 (38861.)
- **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-1λT, 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 P^{32} using the catalytic subunit of cAMP-dependent protein kinase.
- **Host(s):** *E. coli*. The plasmid provides *lacIq* repressor.
- **Selectable marker(s):** Plasmid confers resistance to 100 µg/ml ampicillin.
- **Amplification:** Recommended.

Properties of pGEX vectors • Induction: *tac* promoter inducible with 1 to 5 mM IPTG.

• pGEX-1λT Control Regions:

- Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site

for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-944
* β-lactamase gene region: Promoter: -10: 1308-1313; -35: 1285-1290; Start codon (ATG): 1355; Stop codon (TAA): 2213
* *lacIq* gene region: Start codon (GTG): 3296; Stop codon (TGA): 4376
* Plasmid replication region: Site of replication initiation: 2973; Region necessary for replication: 2280-2976
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1019-997

• pGEX-2T Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-945
* β-lactamase gene region: Promoter: -10: 1309-1314; -35: 1286-1291; Start codon (ATG): 1356; Stop codon (TAA): 2214
* *lacIq* gene region: Start codon (GTG): 3297; Stop codon (TGA): 4377
* Plasmid replication region: Site of replication initiation: 2974; Region necessary for replication: 2281-2977
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1020-998

• pGEX-2TK Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935;
* Coding for kinase recognition site: 936-950
* MCS: 951-966
* β-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
* *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998
Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-3X Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-949
* β-lactamase gene region: Promoter: -10: 1313-1318; -35: 1290-1295; Start codon (ATG): 1360; Stop codon (TAA): 2218
* *lacIq* gene region: Start codon (GTG): 3301; Stop codon (TGA): 4381
* Plasmid replication region: Site of replication initiation: 2978; Region necessary for replication: 2285-2981
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1024-1002

• pGEX-4T-1 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-966
* β-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
* *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-4T-2 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-967
* β-lactamase gene region: Promoter: -10: 1331-1336; -35: 1308-1313; Start codon (ATG): 1378; Stop codon (TAA): 2236
* *lacIq* gene region: Start codon (GTG): 3319; Stop codon (TGA): 4399
* Plasmid replication region: Site of replication initiation: 2996; Region necessary for replication: 2303-2999
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1042-1020

• pGEX-4T-3 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-965
* β-lactamase gene region: Promoter: -10: 1329-1334; -35: 1306-1311; Start codon (ATG): 1376; Stop codon (TAA): 2234
* *lacIq* gene region: Start codon (GTG): 3317; Stop codon (TGA): 4397
* Plasmid replication region: Site of replication initiation: 2994; Region necessary for replication: 2301-2997
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1040-1018

• pGEX-5X-1 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-969

* β-lactamase gene region: Promoter: -10: 1333-1338; -35: 1310-1315; Start codon (ATG): 1380; Stop codon (TAA): 2238
* *lacIq* gene region: Start codon (GTG): 3321; Stop codon (TGA): 4401
* Plasmid replication region: Site of replication initiation: 2998; Region necessary for replication: 2305-3001
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1044-1022

• pGEX-5X-2 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-970
* β-lactamase gene region: Promoter: -10: 1334-1339; -35: 1311-1316; Start codon (ATG): 1381; Stop codon (TAA): 2239
* *lacIq* gene region: Start codon (GTG): 3322; Stop codon (TGA): 4402
* Plasmid replication region: Site of replication initiation: 2999; Region necessary for replication: 2306-3002
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1045-1023

• pGEX-5X-3 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-971
* β-lactamase gene region: Promoter: -10: 1335-1340; -35: 1312-1317; Start codon (ATG): 1382; Stop codon (TAA): 2240
* *lacIq* gene region: Start codon (GTG): 3323; Stop codon (TGA): 4403
* Plasmid replication region: Site of replication initiation: 3000; Region necessary for replication: 2307-3003
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1046-1024

Reference

1. Kaelin, W.G. *et al. Cell* **70**, 351 (1992).

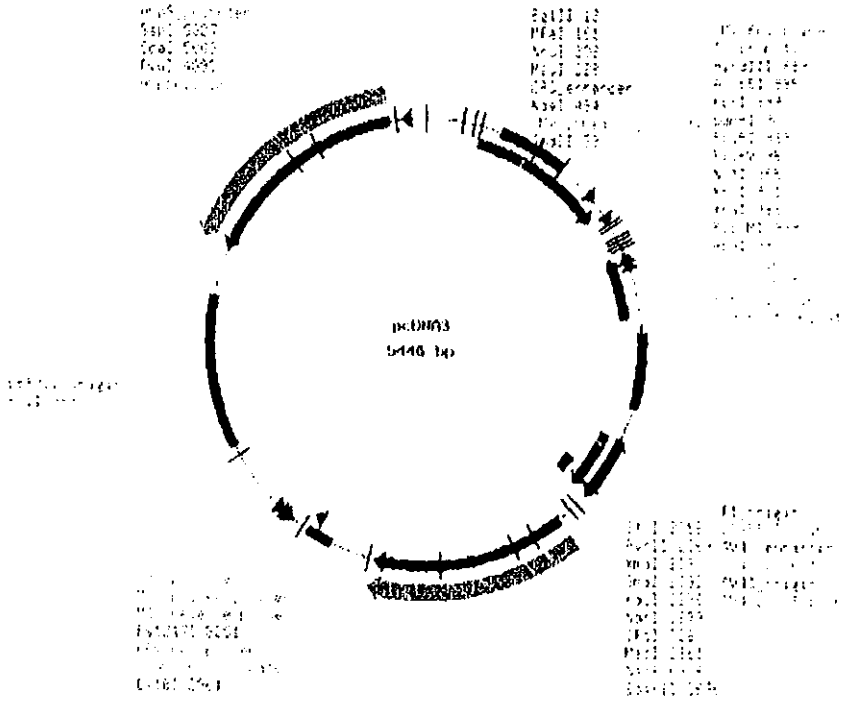
Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

[Close]

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pcDNA3
Source/Vendor	Invitrogen
Plasmid Type	Mammalian expression
Promoter	CMV
Plasmid Size	5446
Sequencing Primer	T7
Bacterial Resistance	Ampicillin
Mammalian Selection	Neomycin
Plasmid Sequence	View Sequence



Certificate of Analysis

Clontech

Adeno-X™ CMV Vector Set

Contents

Catalog No.	Amount	Lot Number
632263 (Not sold separately)	10 rxns	Specified on product label.

Product Information

The Adeno-X CMV Vector Set is supplied with Adeno-X Adenoviral System 3 (CMV) [Cat. No. 632269], which provides a constitutive expression system in an adenoviral vector format. pAdenoX-CMV is a prelinearized, adenoviral vector that is ready for the insertion of your gene of interest via In-Fusion® HD PCR Cloning technology. Simply PCR-amplify your gene and combine it with pAdenoX-CMV in an In-Fusion HD Cloning reaction. In-Fusion HD Cloning is fast, simple, precise, and efficient, making Adeno-X Adenoviral System 3 the most advanced, commercially-available, adenoviral gene delivery tool.

Package Contents

- 10 µl pAdenoX-CMV (Linear) Vector (200 ng/µl)
- 50 µl Adeno-X Screening Primer Mix 3 (10 µM)
- 20 µl Adeno-X Control Fragment (50 ng/µl)

Storage Conditions

- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

Shelf Life

- 1 year from date of receipt under proper storage conditions

Shipping Conditions

- Dry ice (–70°C)

Product User Manuals

User manuals for Clontech products are available for download at **www.clontech.com/manuals**
The following user manual applies to this product:

- Adeno-X Adenoviral System 3 User Manual (PT5177-1)

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: tech@clontech.com

United States/Canada	Asia Pacific	Europe	Japan
800.662.2566	+1.650.919.7300	+33.(0)1.3904.6880	+81.(0)77.543.6116
(PA114266)			

Vector Information

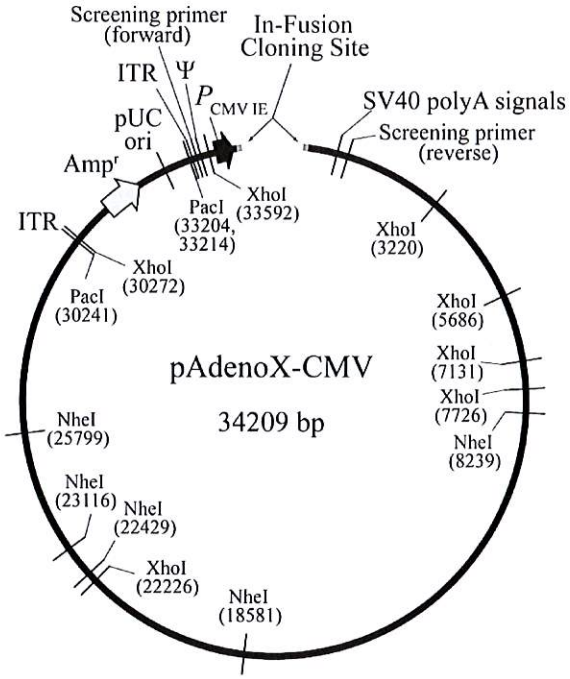


Figure 1. pAdenoX-CMV (Linear) Vector Map.

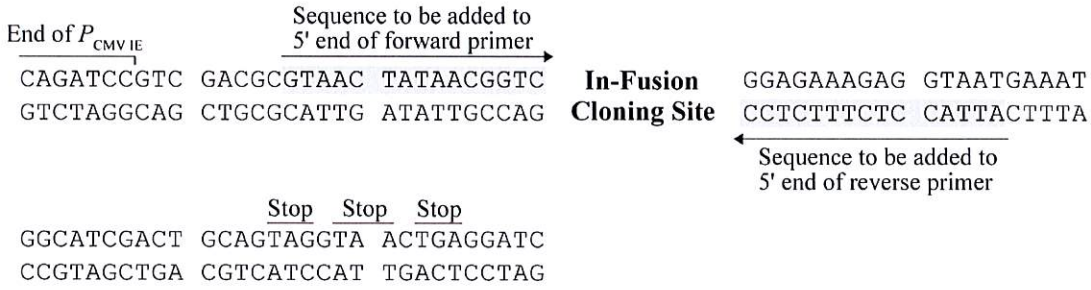


Figure 2. pAdenoX-CMV (Linear) Vector In-Fusion Cloning Site. The shaded regions indicate the 15 nucleotides that need to be added to the 5′ ends of your gene-specific PCR primers in order to create regions of homology with the vector. The sequence at each end is different to allow for directional cloning.

Description

The pAdenoX-CMV (Linear) Vector is a linearized adenoviral expression vector designed to constitutively express a gene of interest in mammalian cells. The ends of the vector serve as the In-Fusion cloning site, allowing direct and rapid cloning of your gene system of interest. Expression of the gene of interest is driven by the constitutively active human cytomegalovirus immediately early promoter ($P_{CMV\ IE}$).

pAdenoX-CMV contains a ΔE1/ΔE3, replication-deficient, type 5 adenovirus genome (Ad5) that is engineered for use in gene delivery and expression studies (1, 2). The Ad5 genome is flanked by inverted terminal repeats (ITR), which are necessary for the replication of adenoviral DNA. The vector also contains a pUC replication origin and an ampicillin resistance gene (Amp^r) for propagation and selection in *E. coli*.

invitrogen

MAX Efficiency® DH10Bac™ Competent Cells

Cat. No. 10361-012

Size: 0.5 ml
Store at -70°C.
Do not store in liquid nitrogen.

Information for European Customers: These cells are genetically modified and carry the pBR322-derived plasmid pMON7124 (*bom*$^+$, *tra*$^-$, *mob*$^-$). As a condition of sale, this product must be used in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Description: MAX Efficiency® DH10Bac™ Competent Cells have been prepared using a patented modification of the procedure of Hanahan (1). MAX Efficiency® DH10Bac™ Competent Cells are used to produce recombinant baculovirus molecules for the expression of eukaryotic proteins (2). MAX Efficiency® DH10Bac™ Competent cells contain the parent bacmid bMON14272 and the helper plasmid pMON7124. The parent bacmid contains a mini-F replicon, the kanamycin resistance gene, an attTn7 site and the *lacZ*α complementation factor. The helper plasmid contains the *tns*ABCD region which supplies the transposition proteins required for insertion of the mini-Tn7 from the donor plasmid into its target site on the parent bacmid. The donor pFastBac™ plasmid carries a Tn7 element containing the gentamicin resitatnce gene, the baculovirus polyhedrin promoter, a multiple cloning site region and the SV40 poolyadenylation signal. A composite bacmid molecule is produced when MAX Efficiency® DH10Bac™ Competent Cells are transformed with a donor pFastBac™ plasmid containing a coding sequence cloned into the multiple cloning site (the composite appears as a white colony on the selective medium described in the transformation procedures). The composite bacmid can then be isolated and transfected into insect cells, resulting in the production of infectious recombinant baculovirus particles.

MAX Efficiency® DH10Bac™ Competent Cells are resistant to the effects of ligase and ligase buffer and can tolerate the addition of small amounts of undiluted ligation reactions (see Note 3). The ϕ80d*lac*ZDM15 marker provides α-complementation of the β-galactosidase gene from the bacmid vector and therefore can be used for blue/white screening of colonies on bacterial plates containing X-gal.

Part no. 10361012.pps Rev date.: 25 October 2006

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ (*ara*, *leu*)7697 *gal*U *gal*K λ⁻ *rps*L *nup*G /pMON14272 / pMON7124

Component	Amount Per Vial
DH10Bac™ Competent Cells	100 µl
pUC19 DNA (0.01 µg/ml)	100 µl

Quality Control: MAX Efficiency® DH10Bac™ Competent Cells consistently yield $> 1 \times 10^8$ transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate $> 1 \times 10^5$ ampicillin-resistant colonies in a 100-µl reaction. A transposition frequency of > 8% (% white colonies) is obtained with 1 ng of pFastBac-gus.

Transformation Procedure: A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. The stock solution of pFastBac-gus (0.2 µg/ml), provided with pFastBac™1 Expression Vector (Cat. No. 10360-014), can be used as a control for the transposition frequency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on wet ice. Place required number of 17 × 100 mm polypropylene tubes (Falcon® 2059) on ice.
2. Gently mix cells, then aliquot 100 µl of competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning to the -70°C freezer. Do not use liquid nitrogen.
4. To determine the transformation efficiency, add 5 µl (50 pg) pUC19 control DNA to one tube containing 100 µl competent cells. To determine the transposition efficiency, add 5 µl (1 ng) pFastBac-gus control DNA to 100 µl of competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. Incubate cells on ice to 30 minutes.
6. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
7. Place on ice for 2 minutes.
8. Add 0.9 ml room temperature S.O.C. Medium (Cat. No.. 15544-034).
9. **For tubes with pFastBac™ constructs**: Shake at 225 rpm (37°C) for 4 hours.
   For tubes with pUC19 control DNA: Shake at 225 rpm (37°C) for 1 hour.

10. Dilute the reaction containing the pFastBac-gus control DNA 1:10, 1:100, and 1:1000 with S.O.C. Medium. Spread 100 µl of this dilution on Luria Agar (Miller's LB Agar) plates that contain kanamycin sulfate, tetracycline, gentamicin, X-gal and IPTG. See Note 4.
11. Dilute the transformations with the pUC19 control DNA 1:100 and plate on LB agar with 100 µg/ml ampicillin.
12. Dilute experimental reactions as necessary and spread 100 to 200 µl of this dilution as described in Step 10.
13. Incubate for no less than 24 hours at 37°C. See notes 6 and 7.

Growth of Transformants for Plasmid Preparations:

MAX Efficiency® DH10Bac™ Cells which have been transformed with pFastBac™-based plasmid should be grown at 37°C overnight in LB broth containing 50 µg/ml kanamycin sulfate, 7 µg/ml gentamicin and 10 µg/ml tetracycline. A 1.5-ml overnight culture inoculated from a single colony will yield sufficient amounts of composite bacmid DNA for several insect cell transfections.

Notes:

1. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately two-fold.
2. Media other than S.O.C. Medium can be used, but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of two- to three-fold (3).
3. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. DH10Bac™ cells can tolerate the addition of up to 1 µl (5 to 50 ng) of an undiluted ligation reaction without a significant loss is transformation efficiency. We have observed that the cells begin to saturate with 10 to 50 ng of DNA (4).
4. Other media may be used for plates; however, the color intensity of the blue/white selection will be reduced.

5. Neither incubation of the plates at a higher temperature nor storage of the plates at 4°C after the 24-hour growth period produces an increase in color intensity.
6. Different size colonies are to be expected. True white colonies (containing composite bacmids) tend to be larger in size. Select the largest, most isolated colonies to avoid cross-contamination.
7. The plates must be incubated at 37°C for not less that 24 hours. Shorter incubation times can result in difficulties interpreting the blue/white selection.
8. Transformation efficiency (CFU/µg):

   $$\frac{\text{CFU in control plate}}{\text{pg pUC 19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

   For example, if 50 pg pUC yields 100 colonies when 100 µl of a 1: 10 dilution is plated, then:

   $$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 2 \times 10^8$$

References:

1. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Luckow, V.A., Lee, S.C., Barry, G.F., and Olins, P.O. (1993) *J. Virol. 67*:4566.
3. Jessee, J. (1988) *Focus® 10:3*, 53.
4. Jessee, J. (1984) *Focus® 6:4*, 5.

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