The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator
Western Ontario (UWO) or in charge of a laboratory/facility w
described in the laboratory or animal work proposed. The for
involving animals carrying zoonotic agents infectious to huma
Public Health Agency of Canada (PHAC) or Canadian Food

Level 2+

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

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

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

PRINCIPAL INVESTIGATOR:	**Stephen Barr**
DEPARTMENT:	**Microbiology & Immunology**
ADDRESS:	**Dental Sciences Building Room 3006b**
PHONE NUMBER:	**x83438**
EMERGENCY PHONE NUMBER(S):	**519-495-1490**
EMAIL:	**stephen.barr@uwo.ca**

Location of experimental work to be carried out :

Building :	**Dental Sciences**	Room(s):	**3006-B**
Building :	**Dental Sciences**	Room(s):	**3006-B2**
Building :		Room(s):	

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **Currently unfunded**

GRANT TITLE(S): **Submitted (abbreviated titles): 1) UWO-ADF Small Grant: Impact of TRIM22 in HIV infection. 2) CIHR: Role of Herc5 in HIV infection. 3) NSERC: Role of HERC4 in spermiogenesis.**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

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

Name	UWO E-mail Address	Date of Biosafety Training
Sherry Xu	**lxu48@uwo.ca**	
Matthew Woods	**mwoods22@uwo.ca**	
Jenna Kelly	**jkelly25@uwo.ca**	
Clayton Hattlmann	**chattlma@uwo.ca**	

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Macon Coleman	mcolem4@uwo.ca	

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

BACTERIAL WORK:

>>>Plasmid preparation- "Cloning strains" (eg. Escherichia coli DH5alpha) are used to propagate our plasmids for purification. Equipment is decontaminated with 10% bleach and washed with soapy water, and/or autoclaved.

>>>Protein expression- "Protein expression strains" (eg. Escherichia coli BL21(DE3)pLysS) are used to express recombinant human proteins for the purpose of purification. Equipment is decontaminated with 10% bleach and washed with soapy water, and/or autoclaved.

MAMMALIAN WORK:

>>>Primary cells and cell lines- A variety of mammalian primary cells and cell lines will be cultured in our Level 2+3 room (DSB3006b2). Cells are maintained in a CO2 incubator with daily sub-culturing for propagation. Cells are manipulated using various transfection techniques (eg. Lipofectamine) in a certified biological safety cabinet. Equipment/supplies/liquid in contact with the cells are decontaminated with 10% bleach and washed with soapy water, and/or autoclaved.

>>>Pseudotyped Human Immunodeficiency Virus-1 (HIV-1)-Replication-incompetent HIV-1 vectors will be used to introduce human genes that are not known to be growth-altering into human cells for stable expression. This will result in the integration of the desired gene into the genome of these cells. The virus is generated by transfecting three plasmids into a human cell line such as 293T. The first plasmid, deltaR9 ("packaging plasmid"), contains the genes to produce the structure of the virus. This construct lacks essential components required for replication such as: it does not contain the LTR sequences (which are required for integration of the virus into the genome), it lacks the genome packaging signal (which means the virus will lack the necessary genetic blue prints to reproduce), it lacks the envelope gene, and it lacks the vpu accessory gene. The second plasmid, VSVg ("envelope plasmid"), codes for an envelope protein from Vesicular Stomatitis Virus to allow entry into cells. This gene is provided in trans and will not be packaged into the genome of new virus (meaning it cannot generate new envelopes in the infected cell). This plasmid simply provides the envelope protein (which is not of HIV origin) in order to allow the virus to get into the target cell. The third plasmid, ppt ("gene delivery plasmid"), contains two LTRs and a cloning site for the insertion of the gene of choice that will be under the control of the CMV promoter. This construct will be integrated into the target cell genome. Since this virus is replication-incompetent, I will perform this procedure in the enclosed tissue culture room (DSB 3006b2) within my lab (DSB 3006b) under Biosafety Level 2+3 conditions with enhanced safety precautions. Equipment/supplies/liquid in contact with virus are decontaminated with 10% bleach and washed with soapy water, and/or autoclaved.

>>>Murine Testis Work- Pending approval from an animal protocol to be submitted in the next couple months, we will obtain murine testis samples for the isolation of spermatid cells. Testis will be manipulated using a dissecting microscope to isolate the seminiferous tubules. The tubules will be minced with scissors, placed in PBS and clarified. Spermatogenic cells will be enriched by flow cytometry analysis and cell sorting using the Robarts facility or Dr. Haeryfars equipment. Cells will be transfected with a plasmid encoding HERC4 and analyzed microscopically for their ability to differentiate into mature sperm. Equipment in contact with murine testis or cells will be decontaminated with 10% bleach and washed with soapy water, and/or autoclaved. Standard decontamination procedures for the flow cytometry equipment will be followed.

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

UWO- ADF Small grant application: TRIM22 is a newly discovered protein in humans that blocks infection by the human immunodeficiency virus (HIV). TRIM22 targets two different parts of the HIV lifecycle by two different ways. One way involves blocking the production of HIV proteins within cells, and the other way prevents the assembly of the virus within cells. The Barr laboratory has discovered that specific mutations in the human TRIM22 gene can inactivate TRIM22. The objective of the proposed project is to determine if genetic differences in the TRIM22 gene can influence HIV infection in patients and their disease progression. The method of investigation will involve reading the genetic code of the TRIM22 gene from a variety of HIV-infected patients at different stages of disease progression. We will then determine if specific genetic differences are associated with protection from, or susceptibility to, HIV infection. Results from the proposed project could provide information for the development of drugs or gene therapy that can enhance the ability of TRIM22 to attack HIV. Our results could also identify a factor for personalized medicine that may help improve patient drug-treatment plans and help predict disease progression in infected individuals.

CIHR: HERC5 is a newly discovered antiviral protein that exhibits potent inhibitory properties towards HIV. The Barr laboratory focuses on the characterization of the molecular mechanism underlying its antiviral activities. Our research involves over-expressing and knocking down HERC5 expression levels in mammalian cells and assessing its effects on mammalian cells and on the ability of HIV to infect these cells (carried out under Level 3 conditions- see Barf UWO-BIO-00224). Our research involves determining how HERC5 inhibits HIV protein synthesis and assembly, and determining the impact of HERC5 gene polymorphisms on HIV infection. We are addressing three specific hypotheses: 1) HERC5 possesses guanine exchange factor activity that disrupts the nuclear export of RNA and hence synthesis of HIV protein; 2) HERC5 conjugates a small protein called ISG15 to target proteins that ultimately interferes with HIV assembly' 3) HERC5 gene polymorphisms exist in the human population to impact a person's susceptibility to HIV infection and disease progression to AIDS. We use a variety of gene transfection and gene transduction (pseudotyped HIV virus) techinques to express our HERC5 constructs in cells. HERC5 or the HERC5 mutant constructs generated in our laboratory are not known to exhibit oncogenic properties. We also perform biochemical analyses on recombinant HERC5 protein to identify and characterize its biological activity. This activity assay is not biohazardous. We will also isolate genomic DNA from healthy and HIV-infected patients in order to sequence the HERC5 gene and identify polymorphisms that may affect HERC5 function. Biosafety level 3 precautions are followed when handling HIV-infected samples (see Barf UWO-BIO-00224).

NSERC: Our focus is on HERC4 since it is the oldest gene of the herc family, from which all 6 herc members were derived. The biological role of human HERC4 is unknown; however murine HERC4 is required for proper maturation of sperm in mice and is associated with male infertility in mice. Our research will advance basic knowledge of the biological function of HERC4, which may prove to be a critical factor in one of the most fundamental cellular processes in biology, the development of sperm. Our research will provide insight into why HERC4 is required for the proper development of sperm and why defective HERC4 is associated with male infertility in mice. We hypothesize that HERC4 is a novel protein that possesses both guanine nucleotide exchange factor activity and E3 ligase activity for a specific and distinct target set of substrate proteins involved in spermiogenesis. We will:1) Identify and characterize biological processes, cellular components and molecular functions targeted by HERC4 using protein pull-down assays and mass spectrometry. We will also determine if HERC4 possesses E3 ligase activity and guanine nucleotide exchange factor activity.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species _____
What is the origin of the microorganism(s)? _____

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Human Immunodeficiency Pseudovirus	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.1**	**NIH AIDS Reagents**	☐ 1 ☐ 2 ☒ 2+ ☐ 3
Escherichia coli DH5alpha	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1**	**Agilent Technologies**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Escherichia coli BL21(DE3)pLysS	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1**	**Agilent Technologies**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Escherichia coli HB101	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1**	**BioRad**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
Escherichia coli Stbl4	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**1**	**Invitrogen**	☒ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

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

Additional Comments: _____

2.0 Cell Culture

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

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒ Yes ☐ No	**Human patients**	Not applicable
Rodent	☒ Yes ☐ No	**Murine testis**	**App. in progress**
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**See next page**		
Rodent	☐ Yes ☒ No			
Non-human primate	☒ Yes ☐ No	**See next page**		
Other (specify)	☐ Yes ☐ No			

****Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)***

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	**Healthy donors**	☐ Yes ☒ Unknown		☐1 ☒2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

Cell Type	Is this cell type used in your work?	Specific cell lines	Containment Level	Viral components	Supplier
Human	Yes	293T	2	Adeno and SV-40 viral sequences	ATCC
	Yes	HOS	1		NIH AIDS Reagents
	Yes	GHOST(3)R3-X4-R5	1		NIH AIDS Reagents
	Yes	HeLa	2	Human Papilloma Virus	ATCC
	Yes	U2OS	1		ATCC
	Yes	143B	1		ATCC
	Yes	Jurkat E6-1	1		ATCC
	Yes	SupT1	1		NIH AIDS Reagents
	Yes	293T Phoenix	2+	Replication-defective HIV-1 packaging genes	S. Kim Lab (UWO)
	Yes	HUT78	1		NIH AIDS Reagents
	Yes	SKSM2	2+	Replication-defective HIV-1	R. Bushman Lab (Upenn)
	Yes	U38-Cat	1		NIH AIDS Reagents
	Yes	CEM-SS	1		NIH AIDS Reagents
	Yes	CEM-GFP	1		NIH AIDS Reagents
	Yes	CEM-T4	1		NIH AIDS Reagents
	Yes	H9	1		NIH AIDS Reagents
	Yes	HeLaCD4-Cat	2	Human Papilloma Virus	NIH AIDS Reagents
	Yes	Hs 181.Tes	1		ATCC
Non-human Primate	Yes	COS-1	2+	Papovavirus	R. Bushman Lab (Upenn)
	Yes	COS-7	2+	SV40 viral sequences	R. Bushman Lab (Upenn)
	Yes	Vero	2+		J. Smiley Lab (U Alberta)
	Yes	Macaca T cell line	2+		NHPRR
Murine	Yes	TM4	1		ATCC
Equine	Yes	EML-3C	1		ATCC

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
Escherichia coli: (DH5alpha, HB101, or Stbl4)	**See attached page**					

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Replication-defective	**Lentiviral**	**NIH AIDS Reagents**	**HERC1-6, TRIM22, HERC1-6 shRNA, TRIM22 shRNA, eGFP shRNA, scrambled shRNA**	**Cells express the transduced genes resulting in over-expression or knockdown (eg. shRNA). No growth-altering activities are known.**

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

4.3.1 Will virus be replication defective? ☒ YES ☐ NO

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

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

5.0 Will genetic sequences from the following be involved?

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

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

Additional Comments: **MLV= murine leukemia virus**

Plasmids	Plasmid Source	Gene transformed/ transfected	Change due to transformation of bacteria?	Change in pathogenicity of bacteria after modification?	Consequences due to transformation of bacteria?
pcDNA3.1	Invitrogen	TRIM22, HERC1-6	None known	None	Plasmid propagation
pCS2	Clontech	HERC1-6	None known	None	Plasmid propagation
pLKO.1	Open Biosystems	shRNA (TRIM22, HERC1-6, eGFP, scrambled)	None known	None	Plasmid propagation
pdeltaR9	R. Bushman (Upenn)	HIV genome minus envelope and Nef	None known	None	Plasmid propagation
pVSVG	R. Bushman (Upenn)	Vesicular stomatitis virus protein G	None known	None	Plasmid propagation
pPPT	R. Bushman (Upenn)	GFP	None known	None	Plasmid propagation
pGag	NIH AIDS Reagents	HIV Gag	None known	None	Plasmid propagation
pEnv	NIH AIDS Reagents	HIV Env	None known	None	Plasmid propagation
pRev	NIH AIDS Reagents	HIV Rev	None known	None	Plasmid propagation
pTat	NIH AIDS Reagents	HIV Tat	None known	None	Plasmid propagation
pNef	NIH AIDS Reagents	HIV Nef	None known	None	Plasmid propagation
pVpu	NIH AIDS Reagents	HIV Vpu	None known	None	Plasmid propagation
pVpr	NIH AIDS Reagents	HIV Vpr	None known	None	Plasmid propagation
p3xFLAG-CMV-10	Sigma	TRIM22, HERC1-6	None known	None	Plasmid propagation
pTRE2hyg-HIV protease	Clontech	HIV protease	None known	None	Plasmid propagation
pLPCX	Clontech	TRIM22	None known	None	Plasmid propagation
pLRCX	Clontech	TRIM22	None known	None	Plasmid propagation
pSIVgorCP2139	NIH AIDS Reagents	SIV genome	None known	None	Plasmid propagation
pSIVagmTan-1	NIH AIDS Reagents	SIV genome	None known	None	Plasmid propagation
pC15CAT	NIH AIDS Reagents	chloramphenicol acetyltransferase	None known	None	Plasmid propagation
pMT-2.HTLV1	G. Dekaban (UWO)	HTLV-1 genome	None known	None	Plasmid propagation
pHTLV1-K30	NIH AIDS Reagents	HTLV-1 genome	None known	None	Plasmid propagation
pTeton	Clontech	TRIM22, HERC1-6	None known	None	Plasmid propagation
pUbiquitin(HA tagged)	K. Chin (Genome Institute of Singapore)	ubiquitin	None known	None	Plasmid propagation
pUbiquitin(His tagged)	K. Chin (Genome Institute of Singapore)	ubiquitin	None known	None	Plasmid propagation
pISG15	K. Chin (Genome Institute of Singapore)	interferon stimulated gene 15	None known	None	Plasmid propagation
pUbe1L	K. Chin (Genome Institute of Singapore)	E1 activating enzyme for ISG15	None known	None	Plasmid propagation
pUbcH8	K. Chin (Genome Institute of Singapore)	E2 activating enzyme for ISG15	None known	None	Plasmid propagation
pUbp43	Open Biosystems	ISG15 deconjugase	None known	None	Plasmid propagation
pGL3	Promega	None-Empty vector control	None known	None	Plasmid propagation

pMLVGag	R. Bushman (Upenn)	MLV Gag Pol	None known	None	Plasmid propagation

Plasmids	Plasmid Source	Gene transformed/ transfected	Change due to transformation of bacteria?	Change in pathogenicity of bacteria after modification?	Consequences due to transformation of bacteria?
pEIAV	NIH AIDS Reagents	EIAV genome	None known	None	Plasmid propagation
pEBFP	Clontech	Blue Fluorescent protein	None known	None	Plasmid propagation
pECFP	Clontech	Cyan fluorescent protein	None known	None	Plasmid propagation
pEGFP-C1	Clontech	Green fluorescent protein	None known	None	Plasmid propagation
pEGFP-N3	Clontech	Green fluorescent protein	None known	None	Plasmid propagation
pET28a	Novagen	TRIM22, HERC5	None known	None	Plasmid propagation
pET41a	Novagen	TRIM22, HERC5	None known	None	Plasmid propagation
pEYFP-N1	Clontech	Yellow fluorescent protein	None known	None	Plasmid propagation

6.0 Human Gene Therapy Trials

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

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

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

7.2 Name of animal species to be used **To be determined**

7.3 AUS protocol # **Not submitted yet**

7.4 List the location(s) for the animal experimentation and housing. **To be determined**

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

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

8.0 Use of Animal species with Zoonotic Hazards

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

8.2 Will live animals be used? ☐ YES ☐ NO

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

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

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

9.0 Biological Toxins and Hormones

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

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

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

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

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

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

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

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

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

11.3 What is the origin of the plant?

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

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

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

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

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

12.0 Import Requirements

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

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

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

12.4 Has the import permit been sent to OHS? ☒ YES, please provide permit # **PHAC:P-17116; CFIA:A-2009-0355** ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Stephen Barr** ***Date:*** **November 1, 2011**

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **November 2010**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **BIO-UWO-00230**

15.0 Procedures to be Followed

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

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
SOPs will be followed. Injured area will be scrubbed with soapy water, rinsed with flowing water. Person will go to Staff Health during work hours or to the emergency room after hours.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Stephen Barr** ***Date:*** **November 1, 2011**

15.4 Additional Comments: _____

16.0 Approvals

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

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

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

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

Special Conditions of Approval:

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

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

Canada

MSDS for SIV

MSDS for SIV is not available.

SIV belongs to the Family *Retroiridae* and Genus *Lentivirus* as is HIV. **Simian immunodeficiency virus** (**SIV**) is a retrovirus that is found, in numerous strains, in primates; the specific strains infecting humans are HIV-1 and HIV-2, the viruses that cause AIDS. Therefore, similar precautions should be taken as with HIV.

MSDS FOR ANIMAL CELL CULTURES (Biosafety Level 1 or 2)

ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as live cells they are potential biohazards.

ATCC Emergency Telephone: (703) 365-2710 (24 hours)
Chemtrec: (800) 424-9300
To be used only in the event of an emergency involving a spill, leak, fire, exposure or accident.

Description

Either frozen or growing cells shipped in liquid cell culture medium (a mixture of components that may include, but is not limited to: inorganic salts, vitamins, amino acids, carbohydrates and other nutrients dissolved in water).

SECTION I
Hazardous Ingredients

Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
Physical data

Pink or red aqueous liquid

SECTION III
Health hazards

For Biosafety Level 1 Cell Lines

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

For Biosafety Level 2 Cell Lines

This cell line is known to contain an agent that requires handling at Biosafety Level 2 containment [U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999)]. These agents have been associated with human disease. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

SECTION IV
Fire and explosion

Not applicable

Office of Laboratory Security

MSDS

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: ***Human Immunodeficiency Virus***

SYNONYM OR CROSS REFERENCE: HIV, AIDS, Acquired Immune Deficiency Syndrome, HTLV III LAV

CHARACTERISTICS: Retroviridae (Lentivirus); ss RNA, enveloped icosahedral nucleocapsid, glycoprotein envelope, reverse transcriptase

SECTION II - HEALTH HAZARD

PATHOGENICITY: Insidious onset with non-specific symptoms such as lymphadenopathy, anorexia, chronic diarrhea, weight loss, fever, and fatigue; opportunistic infections and malignant diseases without a known cause for immune deficiency

EPIDEMIOLOGY: First reported in 1981; cases recorded in Americas, Europe, Africa and many other areas; patient categories - homosexually or bisexually active men, drug abusers, Haitian/African emigrants, hemophiliacs, sexual partners of men and women in these categories, infants born to parents in this category

HOST RANGE: Humans

INFECTIOUS DOSE: Unknown

MODE OF TRANSMISSION: Transmitted from person to person through direct exposure to infected body fluids (blood, semen) sexual contact, sharing unclean needles etc.; transplacental transfer can occur

INCUBATION PERIOD: Epidemiologic evidence suggests that duration from exposure to onset of symptoms has a minimum range from 6 months to more than 7 years

COMMUNICABILITY: Period of communicability extends from asymptomatic period through appearance of opportunistic diseases

SECTION III - DISSEMINATION

RESERVOIR: Humans

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Several reverse transscriptase and protease inhibitors now licensed

SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to many disinfectants - 1% sodium hypochlorite, 2% glutaraldehyde, formaldehyde, ethanol

PHYSICAL INACTIVATION: Effectiveness of 56·C - 60·C heat in destroying HIV in serum not certain, however, heating small volumes of serum for 30 min at 56·C before serologic testing reduces residual infectivity to below detectable levels

SURVIVAL OUTSIDE HOST: Drying in environment causes rapid (within several hours) 90-99% reduction in HIV concentration

SECTION V - MEDICAL

SURVEILLANCE: Serological monitoring for evidence of HIV infection

FIRST AID/TREATMENT: Specific measures for the opportunistic diseases that result from AIDS; "Cocktail" multidrug treatment for HIV

IMMUNIZATION: None available

PROPHYLAXIS: Experimental prophylaxis with AZT/DDI or other appropriate drug

SECTION VI - LABORATORY HAZARDS

LABORATORY-ACQUIRED INFECTIONS: 5 reported laboratory acquired infections with HIV (splashing of infected materials, inapparent skin exposure, puncture wounds); 18 reported cases in health care workers worldwide

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Info on Cell Line(s)

ATCC Advanced Catalog Search » **Product Details**

Product Description

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Cell Biology

ATCC® Number: CRL-8303™ Order this Item

Price: $329.00

Designations: 143B

Depositors: Wistar Institute

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: mixed

Source:
Organ: bone
Disease: osteosarcoma

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

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 10,13
D5S818: 13
D7S820: 11,12
THO1: 6
TPOX: 11
vWA: 18

Age: 13 year old

Gender: female

Ethnicity: Caucasian

Comments: Thymidine kinase negative (TK-).
This is a human osteosarcoma cell line.

Propagation: **ATCC complete growth medium:** Minimum essential medium (Eagle) in Earle's BSS with 0.015 mg/ml 5-bromo-2'-deoxyuridine, 90%; fetal bovine serum, 10%
Temperature: 37.0°C

Subculturing: **Medium Renewal:** 2 to 3 times per week
Remove medium, rinse with fresh 0.25% trypsin, 0.02% EDTA solution and allow the cells to sit at room temperature (or at 37C) until they detach (about 10 minutes). Add fresh medium, aspirate and dispense into new flasks.

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

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NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
Bldg. 6, Suite 200
Germantown, MD 20874
USA

Phone: 240 686-4740
Fax: 301-515-4015
www.aidsreagent.org

DATA SHEET

Reagent: U38

Catalog Number: 1297

Lot Number: 2 95082

Provided: 6.3 x 10^6 cells/vial.

Propagation Medium: RPMI 1640, 90%; fetal bovine serum, 10%.

Freeze Medium: RPMI 1640, 70%; fetal bovine serum, 20%; DMSO, 10%.

Growth Characteristics: Split twice weekly 1:10. U38 cells are stable and do not need to be maintained in selection medium. If growth in selection medium is desired, propagation medium containing 700 µg/ml G418 should be used. Wash the thawed cells in propagation medium and centrifuge for 10 minutes at 1000 rpm before seeding the cells in a culture flask.

Sterility: Negative for bacteria, fungi, and mycoplasma.

Special Characteristics: U38[1] is a U937 derivative that contains stably integrated, silent copies of the HIV-1 LTR promoter linked to the CAT gene. This cell line was generated by infection of U937 cells with a helper-free recombinant retroviral vector containing the HIV-1 LTR-CAT gene construct. U38 was selected in geneticin (G418) under limiting dilution and is a sensitive indicator cell line for HIV-1 Tat. When infected by HIV-1, U38 produces high levels of chloramphenicol acetyl transferase (CAT)[1,2]. Morphology is monocyte-like. Contains LTR sequences to +80 in the R region. Contains the entire U3 region, but lacks U5 sequences.

Recommended Storage: Liquid nitrogen.

Contributor: Dr. Barbara K. Felber and Dr. George N. Pavlakis.

References:

[1]Felber BK, Pavlakis GN. A quantitative bioassay for HIV-1 based on trans-activation. *Science* **239:**184-187, 1988.

[2]Schwartz S, Felber BK, Fenyo EM, Pavlakis GN. Rapidly and slowly replicating human immunodeficiency virus type 1 isolates can be distinguished according to target-cell tropism in T-cell and monocyte cell lines. *Proc Natl Acad Sci USA* **86:**7200-7203, 1989.

Note: Acknowledgment for publications should read "The following reagent was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: U38 from Dr. Barbara K. Felber and Dr. George N. Pavlakis." Also include the references cited above in any publications.

An NCI patent application has been filed on the use of the cell line U38. Corporate requests should be directed in writing to: B.K. Felber or G.N. Pavlakis, National Cancer Institute, FCRDC, ABL-Basic Research Program, P.O. Box B/Building 539, Room 121, Frederick, Maryland 21702-1201. Phone: (301) 846-1474, FAX (301) 846-5991.

Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 PGM1, 2 PGM3, 1
Age:	15 years
Gender:	female
Ethnicity:	Caucasian
Comments:	J. Ponten and E. Saksela derived this line (originally 2T) in 1964 from a moderately differentiated sarcoma of the tibia of a 15 year old girl. Viruses were not detected during co-cultivation with WI-38 cells or in CF tests against SV40, RSV or adenoviruses. Mycoplasma contamination was detected and eliminated in 1972.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated McCoy's 5a Medium Modified, Catalog No. 30-2007. 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:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%
Related Products:	recommended serum:ATCC 30-2020
References:	22237: Heldin CH, et al. A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF A-chains. Nature 319: 511-514, 1986. PubMed: 3456080 22509: Ponten J, Saksela E. Two established in vitro cell lines from human mesenchymal tumours. Int. J. Cancer 2: 434-447, 1967. PubMed: 6081590 23011: Raile K, et al. Human osteosarcoma (U-2 OS) cells express both insulin-like growth factor-I (IGF-I) receptors and insulin-like growth factor-II/mannose-6- phosphate (IGF-II/M6P) receptors and synthesize IGF-II: autocrine growth stimulation by IGF-II via the IGF-I receptor. J. Cell. Physiol. 159: 531-541, 1994. PubMed: 8188767 32288: Landers JE, et al. Translational enhancement of mdm2 oncogene expression in human tumor cells containing a stabilized wild-type p53 protein. Cancer Res. 57: 3562-3568, 1997. PubMed: 9270029 32308: Moradpour D, et al. Characterization of cell lines allowing titghtly regulated expression of heapatitis C virus core protein. Virology 222: 51-63, 1996. PubMed: 8806487

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Back to my Search

3. HAZARDS IDENTIFICATION

Emergency Overview

The product contains no substances which at their given concentration, are considered to be hazardous to health
May be harmful if swallowed
May cause skin and eye irritation in susceptible persons

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

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

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

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

5. FIRE-FIGHTING MEASURES

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

6. ACCIDENTAL RELEASE MEASURES

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

11. TOXICOLOGICAL INFORMATION

Acute toxicity

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

Principle Routes of Exposure/ Potential Health effects

Eyes May cause eye irritation with susceptible persons.
Skin May cause skin irritation in susceptible persons.
Inhalation No information available
Ingestion May be harmful if swallowed.

Specific effects (Long Term Effects)

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

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

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

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

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

15. REGULATORY INFORMATION

International Inventories

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

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Product Description

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Cell Biology

ATCC® Number: CRL-1715™ Order this Item

Price: $329.00

Designations: TM4

Depositors: JP Mather

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: epithelial

Source:
Organ: testis
Disease: normal
Cell Type: Sertoli cell;

Cellular Products:
retinol binding protein
tissue plasminogen activator
transferrin

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 (Roche Transfection Reagents)

Receptors:
follicle stimulating hormone (FSH), expressed
androgen receptor, expressed
progesterone receptor, expressed

Tumorigenic: No

Antigen Expression: H-Y antigen; Mus musculus, expressed

Age: 11 to 13 days

Gender: male

Comments: The TM4 cell line is reported to respond to FSH with an increase in cAMP production, but to not respond to luteinizing hormone (LH). The FSH responsiveness is much reduced compared to primary sertoli cell cultures. Constitutive plasminogen activator production is reported to be low, but is stimulated by FSH and, to a greater extent, by retinoic acid.
Tested and found negative for ectromelia virus (mousepox).

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Related Cell Culture Products

Technical Data Sheet

Reagent: Cynomolgus T cell line

Clone: HSC-F

Lot: Dec 2007

Species: *Macaca fascicularis*

Description: Immortalized cell line derived by transformation of cynomolgus monkey fetal splenocytes with *Herpesvirus saimiri (1)*. IL-2-independent. Cell line kindly provided by Dr. Hirofumi Akari, Tsukuba Primate Research Center and the Health Science Research Resources Bank, Osaka, Japan

Supplied as: 10^7 cells in 90% FBS, 10% DMSO, shipped on dry ice

Medium: 90% RPMI 1640 with L-glutamine,10% FBS and penn/strep

Doubling time: 30 hours

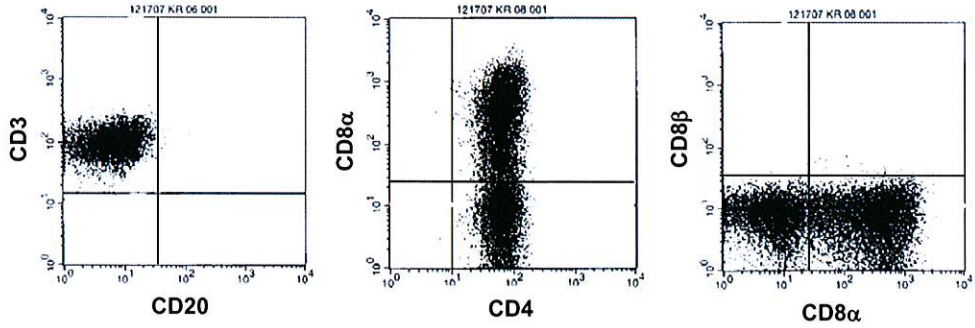
Instructions for propagation: Cells grow as small clumps largely in suspension culture. Split cultures 1-2 times weekly as needed.

BIOHAZARD:

It is unknown whether this cell line is productively infected with *Herpesvirus saimiri. H. saimiri* has been classified as NIH Risk Group I*.

*Source: Biosafety in Microbiological and Biomedical Laboratories, 5th Edition. U.S Government Printing Office, Washington: 2007.

Immunophenotype: CD3+ (100%), CD4+ (100%), CD8α+ (~50%), CD8β– (0%), CD20– (0%)



References: (1) Akari H, Nam KH, Mori K, Otani I, Shibata H, Adachi A, Terao K, Yoshikawa Y. Effects of SIVmac infection on peripheral blood CD4+CD8+ T lymphocytes in cynomolgus macaques. *Clin Immunol. 1999; 91(3):321-9.*

For Research Use Only- All reagents are to be used in accordance with the terms of the Resource registration agreement.

NIH NONHUMAN PRIMATE REAGENT RESOURCE
http://nhpreagents.bidmc.harvard.edu
Beth Israel Deaconess Medical Center
Research East 113
330 Brookline Avenue
Boston, MA 02215
617-667-4583

The NIH Nonhuman Primate Reagent Resource is sponsored by the National Center for Research Resources and the National Institute of Allergy and Infectious Diseases, NIH

Comments:

This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line. [1609]
The Jurkat cell line was established from the peripheral blood of a 14 year old boy by Schneider et al., and was originally designated JM. [50685] [112530]
Clone E6-1 cells produce large amounts of IL-2 after stimulation with phorbol esters and either lectins or monoclonal antibodies against the T3 antigen (both types of stimulants are needed to induce IL-2 production. [1609]
The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free. [1609]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C

Subculturing:

Protocol: Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 X 10(5) viable cells/ml. Do not allow the cell density to exceed 3 X 10(6) cells/ml.
Interval: Maintain cultures at a cell concentraion between between 1 X 10(5) and 1 X 10(6) viable cells/ml.
Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)

Preservation:

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

Doubling Time:

48 hrs

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020
derivative:ATCC CRL-1990
derivative:ATCC CRL-2063
derivative:ATCC TIB-153

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Cell Biology

ATCC® Number: TIB-161™ Order this Item

Price: $279.00

Designations: HuT 78

Depositors: AF Gazdar

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: suspension

Organism: *Homo sapiens* (human)

Morphology: lymphoblast

PHOTO

Source: **Disease:** Sezary Syndrome
Cell Type: cutaneous T lymphocyte;

Cellular Products: interleukin 2 [1140]
tumor necrosis factor alpha (TNF alpha) [23420]

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

Receptors: interleukin 2 (IL-2), expressed [1140]

Tumorigenic: Yes

Antigen Expression: CD4; Homo sapiens [22610]

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

Age: 53 years adult

Gender: male

Ethnicity: Caucasian

Related Links

NCBI Entrez Search

Cell Micrograph

Make a Deposit

Frequently Asked Questions

Material Transfer Agreement

Technical Support

Related Cell Culture Products

Login Required

Product Information Sheet

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Cell Biology

ATCC® Number:	**CRL-7131™** Order this Item	**Price:**	**$429.00**
Designations:	Hs 181.Tes		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	fibroblast		
Source:	**Organ:** testis **Disease:** normal		
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.		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 13 D13S317: 8,13 D16S539: 12 D5S818: 8,12 D7S820: 10,11 THO1: 6,9 TPOX: 8,11 vWA: 19		
Cytogenetic Analysis:	modal number = 46; range = 45 to 47		
Age:	14 weeks gestation		
Gender:	male		
Ethnicity:	Caucasian		
Comments:	Part of the NBL Cell Line Collection. This cell line is neither produced nor fully characterized by ATCC . We do not guarantee that it will maintain a specific morphology, purity, or any other property upon passage. Please see the NBL Repository description.		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C		

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ATCC® Number: CRL-1543™ Order this Item

Price: $329.00

Designations: HOS

Depositors: JS Rhim

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: mixed, fibroblast and epithelial like cells

Source: **Organ:** bone
Disease: osteosarcoma

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.

DNA Profile (STR):
Amelogenin: X
CSF1PO: 12
D13S317: 12
D16S539: 10,13
D5S818: 13
D7S820: 11,12
THO1: 6
TPOX: 8,11
vWA: 18

Isoenzymes: G6PD, B

Age: 13 years

Gender: female

Ethnicity: Caucasian

Comments: HOS cells exhibit flat morphology, low saturation density, low plating efficiency in soft agar and are sensitive to chemical and viral transformation.

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

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ATCC® Number: CCL-2™ Order this Item

Price: $279.00

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: adenocarcinoma
Cell Type: epithelial

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

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References:

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Comments:

The EML-3C cell line was established in 2005 from isolated peripheral blood mononuclear cells of a two-year-old male Portuguese Garrano horse. These cells possess functional properties of macrophages such as non-specific esterase (NSE) activity, are able to phagocytose fluorescent bioparticles and produce nitrites in response to lipopolysaccharide (LPS) stimulation. The EML-3C cell line expresses the EIA V receptor for cellular entry (ELR1) and supports replication of the virulent equine infectious anemia virus (EIA V_{pv}). This cell line can be used as a valuable tool for studying equine macrophage functions, lentivirus infection, and the equine immune system. [16173840]

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 500 ml of the base medium:

- fetal bovine serum (FBS) to a final concentration of 10%
  - horse serum (HS) to a final concentration of 10%
    - 0.1 mM non-essential amino acids

Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO_2), 5%

Subculturing:

Protocol: Volumes used in this protocol are for 75 cm^2 flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with Ca++/Mg++ free Dulbecco's phosphate-buffered saline (D-PBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37.0°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Transfer cell suspension to a centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes. Discard supernatant.
6. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10^4 to 4 X 10^4 viable cells/cm^2 is recommended.
7. Incubate cultures at 37.0°C.
   Subcultivation ratio: A subcultivation ratio of 1:4 to 1:8 is recommended.
   Medium renewal: Every 2 to 3 days

Preservation:

Freeze medium: complete growth medium, 95%; DMSO, 5%
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional serum described under ATCC Medium): ATCC 30-2002
Recommended fetal bovine serum: ATCC 30-2020
Recommended horse serum: ATCC 30-2040
Trypsin EDTA Solution: ATCC 30-2101
Phosphate-buffered saline: ATCC 30-2200
Cell culture tested DMSO: ATCC 4-X
MEM Non-Essential Amino Acid Solution, 100x, ATCC 30-2116

References:

16173840: Isabel Fidalgo-Carvalho et al. Characterization of an equine macrophage cell line: application to studies of EIAV infection. Vetenary Microbiol. 136 (1-2): 8?19, 2009. PubMed: 19038510

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Subculturing:

Protocol:

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

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

Preservation:

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

Related Products:

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

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ATCC® Number: CRL-1650™ Order this Item **Price:** $279.00

Designations: COS-1

Depositors: Y Gluzman

Biosafety Level: 2 [Cells Contain PAPOVAVIRUS]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Cercopithecus aethiops*

Morphology: fibroblast

Source: **Organ:** kidney
Cell Type: SV40 transformed

Cellular Products: T antigen

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 (Roche Transfection Reagents)

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

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

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NIH AIDS Research & Reference Reagent Program
20301 Century Boulevard
Building 6, Suite 200
Germantown, MD 20874
USA

Phone: 240-686-4740
Fax: 301-515-4015
aidsreagent.org

DATA SHEET

Reagent:	CEM-SS
Catalog Number:	776
Lot Number:	15 070569
Release Category:	C
Provided:	1.3 x 10^7 cells/mL. Viability is 96%.
Propagation Medium:	RPMI 1640, 89%; PSN antibiotics (Gibco), 1%; fetal bovine serum, 10%.
Freeze Medium:	RPMI 1640, 66%; fetal bovine serum, 27%; DMSO, 7%.
Growth Characteristics:	These cells double approximately every 1-2 days and grow as a suspension of single or small (3-10 cell) aggregates. The cells are optimally maintained on a rocker platform or roller bottle apparatus and can be split at 1:20 one to two times per week.
Morphology:	Generally a round, individual, slightly refractile cell population that occasionally forms small aggregates as observed under normal culture conditions. Small numbers of individual highly refractile karyocytomegalic cells may also be observed.
Special Characteristics:	These cells have been cloned for both poly-L-lysine induced adherence to microtiter plates and viral-induced syncytial/fusigenic sensitivity following infection with either cell-free or cell-associated HIV-1 and HIV-2. Cells are negative for any virus including human retroviruses as determined by electron microscopy and reverse transcriptase analysis. They can be used for virus production, aspects of HIV-1 cell fusion and molecular biology studies and for the analysis of infectivity, antiviral agents and neutralizing antibodies in the assays referenced below. CEM-SS Microtiter Syncytial-Forming Assay

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ATCC® Number: CRL-11268™ Order this Item **Price:** $279.00

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 ATCC by 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]
DNA Profile (STR):	Amelogenin: X CSF1PO: 11, 12 D13S317: 12, 14 D16S539: 9, 13 D5S818: 8, 9 D7S820: 11 THO1: 7, 9.3 TPOX: 11 vWA: 16, 18, 19
Age:	fetus

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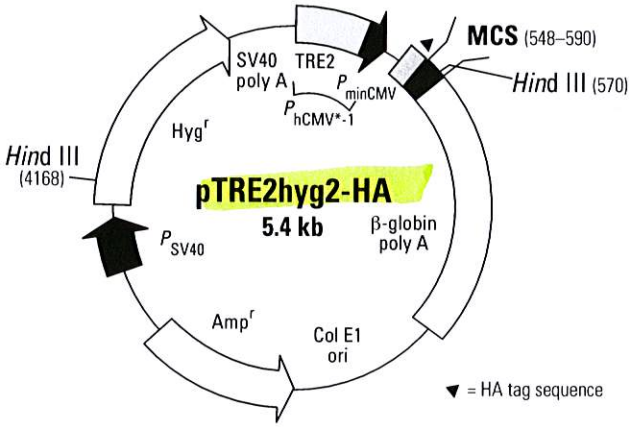
Plasmid Maps



510 520 530
ATG TAC CCA TAC GAT GTT CCA GAT TAC GCT CTT
HA tag

540 550 560 570 580 590 600
ATG GCC ACT GAC GCG TTG CTA GCG CAG CTG GAA GCT TAT CGA TGC GGC CGC GTC GAC GAT ATC
MluI **NheI** **ClaI** **NotI** **AccI/SalI** **EcoRV**

Map, HA tag Sequence and Multiple Cloning Site (MCS) of pTRE2hyg2-HA Vector. Unique restriction sites are in bold.

Description

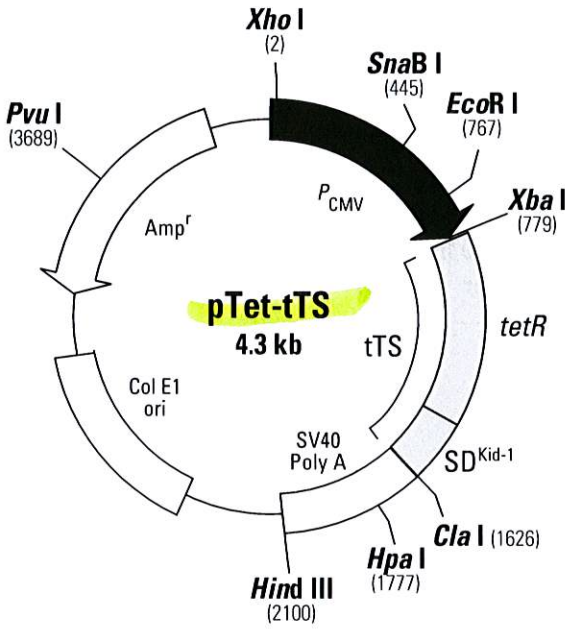
pTRE2hyg2-HA is a Tet responsive vector that expresses a gene of interest bearing a hemagglutinin tag (HA) for use with Tet-On® and Tet-Off® Gene Expression Systems and Tet-On and Tet-Off Cell Lines (1–3). The Tet Expression Systems and Cell Lines give researchers ready access to the tetracycline-regulated expression systems described by Gossen & Bujard (2; Tet-Off) and Gossen *et al.* (3; Tet-On). pTRE2hyg2-HA contains an MCS immediately downstream of a sequence that codes for the HA tag. Transcription is regulated by the Tet-responsive promoter P_{hCMV^*-1}. cDNAs or genes inserted into the MCS will be responsive to the tTA and rtTA regulatory proteins in the Tet-Off and Tet-On systems, respectively. P_{hCMV^*-1} contains the Tet response element (TRE), which consists of seven copies of the 19-bp tet operator sequence (*tetO*). The TRE element is just upstream of the minimal CMV promoter (P_{minCMV}), which lacks the enhancer that is part of the complete CMV promoter. Consequently, P_{hCMV^*-1} is silent in the absence of binding of tTA or rtTA to the *tetO* sequences. pTRE-2hyg2-HA also contains the hygromycin resistance gene for direct selection of stable transformants. The parental vector pTRE2 was originally described as pUHD10-3 in reference 4.

The pTRE2hyg2-HA-Luc Control Vector, packaged with the pTRE2hyg2-HA Vector, contains an additional 1653 bp encoding firefly luciferase inserted into the MCS. This vector can be used as a reporter of induction efficiency using standard luciferase detection reagents. It is not intended for use as a cloning vector.

(PR26138; published 27 August 2002)

pTet-tTS Vector Information PT3334-5

GenBank Accession No.: Submission in progress. Cat. No. 631011



Restriction map of pTet-tTS Vector. All restriction sites shown are unique.

Description:

The pTet-tTS Vector, designed for use with the Tet-On™ Gene Expression System, prevents unregulated gene expression in the absence of the inducing agent Doxycycline (Dox).

pTet-tTS helps overcome one of the major limitations of regulated gene expression in mammalian systems: low-level background expression. In transient transfections, background expression can result from the high copy number of the introduced plasmid and lack of chromatin repression. In stable cell lines, the level of background expression is dependent on where the gene of interest integrates into the genome. If the expression construct integrates too close to an enhancer element, for instance, unregulated expression may occur.

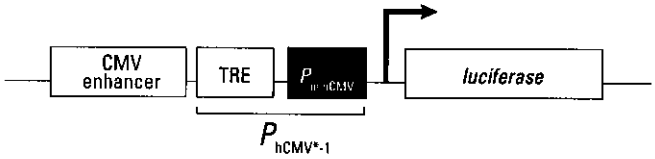
pTet-tTS encodes the tetracycline-controlled transcriptional silencer (tTS), which is a fusion of the tet repressor protein (TetR) and the KRAB-AB silencing domain of the Kid-1 protein (SD^{Kid-1}), a powerful transcriptional repressor (1, 2). In the *absence* of Dox, tTS binds to the *tetO* sequence in the tet-response element (TRE) region of the Tet response plasmid (pTRE2 or pRevTRE) and blocks expression of the gene of interest. As Dox is added to the culture medium, the tTS dissociates from the TRE, relieving transcriptional suppression. At sufficient Dox concentrations, the rtTA transactivator encoded by the pTet-On™ Vector binds to the TRE, thus activating expression of the gene of interest. By silencing unregulated transcription in the *absence* of Dox, the tTS provides complete on/off control of gene expression in either stable or transient systems—regardless of integration site or copy number of the response plasmid.

Use:

tTS will bind to the TRE in the presence of 0–10 ng/ml of Dox. It begins to dissociate from the TRE at concentrations >10 ng/ml. rtTA will bind to the TRE at >100 ng/ml of Dox. We recommend a working Dox concentration of 1 µg/ml for inducing gene expression.

pTet-tTS can be introduced in one of three ways: during the establishment of a Tet-On cell line; after establishing a Tet-On cell line and before introducing the response plasmid; or during introduction of the response plasmid. The last option is recommended if you are introducing a potentially toxic gene. pTet-tTS should only be used with the Tet-On Gene Expression System. It is not compatible for use with the Tet-Off™ System.

(PR651793; published 12 May 2006)



ptTS-Control Vector

Schematic map of ptTS-Control Vector.

References:

1. Freundlieb, S., *et al.* (1999) *J. Gene Med.* **1**:4–12.
2. Witzgall, R., *et al.* (1994) *Proc. Natl. Acad. Sci. USA* **91**:4514–4518.

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http://www.tetsystems.com/main_inquiry.htm

Location of Features

- Fragment containing P_{CMV} : 86–673
- reverse tetacycline-responsive transcriptional activator (rtTA): 774–1781
- Col E1 origin of replication: 2604–3247
- Ampicillin resistance gene:
  β-lactamase coding sequences: 4255–3395
- Fragment containing the SV40 poly A signal: 1797–2254
- Neomycin/kanamycin resistance gene: 6462–5668
- SV40 promoter (P_{SV40}) controlling expression of neomycin/kanamycin resistance gene: 7125–6782.

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 µg/ml) on *E. coli* hosts.
- *E. coli* replication origin: Col E1

References

1. Tet Expression Systems and Cell Lines (July 1996) *Clontechniques* **XI**(3):2–5.
2. Gossen, M. & Bujard, H. (1992) *Proc. Natl. Acad. Sci. USA* **89**:5547–5551.
3. Gossen, M., *et al.* (1995) *Science* **268**:1766–1769.
4. Resnitzky, D., *et al.* (1994) *Mol. Cell. Biol.* **14**:1669-1679.

Note:

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

Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Puromycin resistance gene (Puro^r):
  Start codon: 1566–1568; stop codon: 2163–2165
- Immediate early CMV promoter ($P_{CMV\ IE}$): 2338–2868
- Multiple Cloning Site (MCS): 2888–2964
- 3' MoMuLV LTR: 3004–3597
- Col E1 origin of replication:
  Site of replication initiation: 4266
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5886–5884; stop codon: 5028–5026

Sequencing Primer Locations

- pLNCX Seq/PCR Primers (#K1060-F)
  5' primer (2844–2868): 5'-AGCTCGTTTAGTGAACCGTCAGATC-3'
  3' primer (3026–3001): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*

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

References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
4. Mann, R., *et al.* (1983) *Cell* **33**:153–159.
5. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**:2895–2902.
6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
7. Miller, A. D. & Chen, F. (1996) *J. Virol.* **70**:5564–5571.

Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

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

Location of Features

- 5' MoMuSV LTR: 1–589
- Ψ^+ (extended packaging signal): 659–1468
  Mutated *gag* (ATG->TAG): 1049–1051
- Puromycin resistance gene (Puro^r):
  Start codon: 1566–1568; stop codon: 2163–2165
- Immediate early CMV promoter ($P_{CMV\ IE}$): 2338–2868
- Multiple Cloning Site (MCS): 2888–2964
- 3' MoMuLV LTR: 3004–3597
- Col E1 origin of replication:
  Site of replication initiation: 4266
- Ampicillin resistance gene (β-lactamase):
  Start codon: 5886–5884; stop codon: 5028–5026

Sequencing Primer Locations

- pLNCX Seq/PCR Primers (#K1060-F)
  5' primer (2844–2868): 5'-AGCTCGTTTAGTGAACCGTCAGATC-3'
  3' primer (3026–3001): 5'-ACCTACAGGTGGGGTCTTTCATTCCC-3'

Propagation in *E. coli*

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

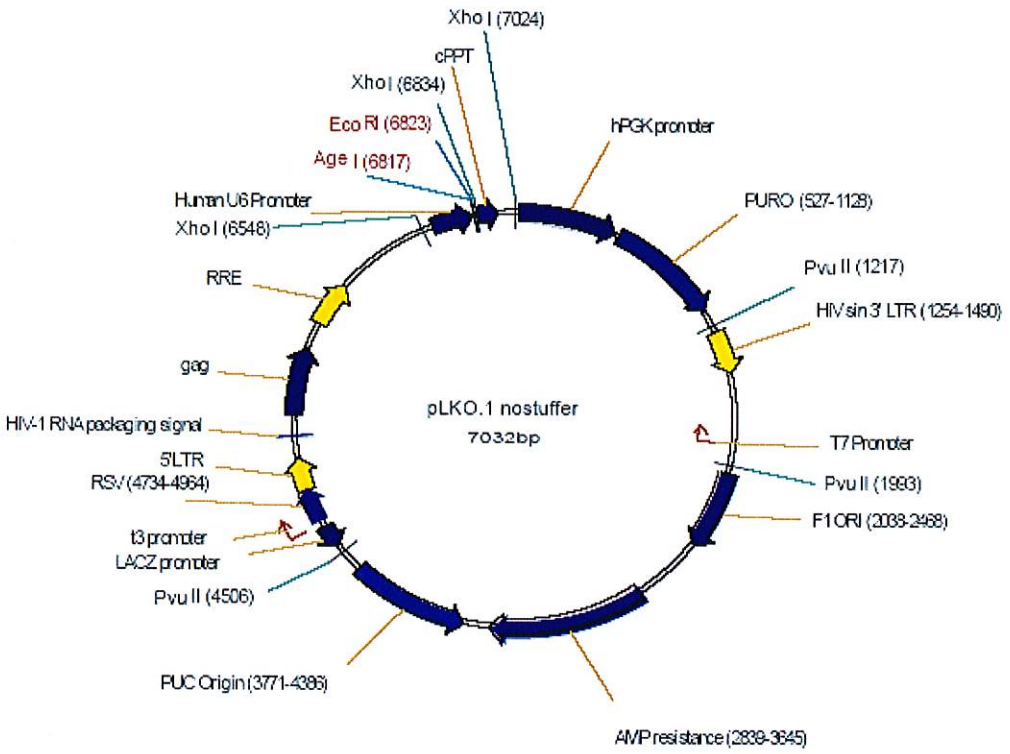
References

1. Coffin, J. M. & Varmus, H. E., Eds. (1996) *Retroviruses* (Cold Spring Harbor Laboratory Press, NY).
2. Ausubel, F. M., *et al.* (1994) *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. & John Wiley & Sons, Inc.).
3. Miller, A. D. & Rosman, G. J. (1989) *BioTechniques* **7**:980–990.
4. Mann, R., *et al.* (1983) *Cell* **33**:153–159.
5. Miller, A. D. & Buttimore, C. (1986) *Mol. Cell. Biol.* **6**:2895–2902.
6. Morgenstern, J. P. & Land, H. (1990) *Nucleic Acids Res.* **18**:3587–3590.
7. Miller, A. D. & Chen, F. (1996) *J. Virol.* **70**:5564–5571.

Notes: The viral supernatants produced by this retroviral vector could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply.

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

Figure 1: Vector Map of pLKO.1



VECTOR ELEMENT	UTILITY
Human U6 Promoter	RNA generated with four uridine overhangs at each 3' end
hPGK	Human phosphoglycerate kinase promoter
PuroR	Puromycin mammalian selectable marker
3' SIN LTR	3' Self inactivating long terminal repeat
f1 ori	f1 origin of replication
AmpR	Ampicillin bacterial selectable marker.
5'LTR	5' long terminal repeat
RRE	Rev response element
cPPT	Central polypurine tract

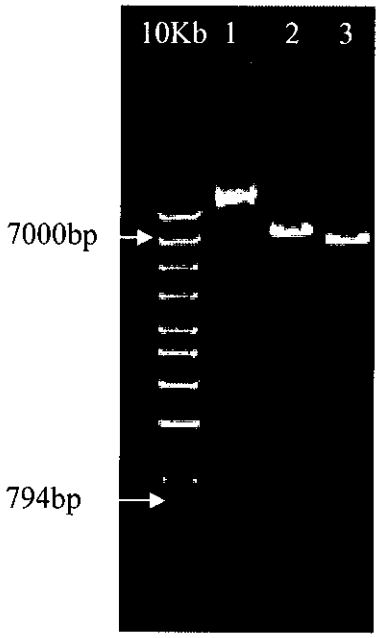


Figure 2. The 1% agarose gel above contains ~10kb ladder followed by undigested sample and restriction digests of pLKO.1 vector (labeled 1-3), The lanes are loaded as follows:1 - Uncut plasmid 2 - Cut with BamHI. Expected to linearize at 7032bp. 3 - Cut with BamHI and NdeI. Band sizes of 6238bp and 794bp expected.

gtcaagctctaaatcgggggctcccttagggttccgatttagtgctttacggcacctcgaccccaaaaaa
cttgattagggtgatggttcacgtagtgggccatcgccctgatagacggtttttcgccctttgacgttgga
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gagtaataaatctctggaacagatttggaatcacacgacctggatggagtgggacagagaaattaacaatt

Useful References:

Stewart, S.A., *et al.*, Lentivirus-delivered stable gene silencing by RNAi in primary cells. RNA, **9**, 493-501 (2003).

Zufferey R, *et al.*, Multiply attenuated lentiviral vector achieves efficient gene delivery *in vivo*. Nat. Biotechnol. **15**, 871-85 (1997).

Zufferey R, *et al.*, Self-inactivating lentivirus vector for safe and efficient *in vivo* gene delivery, J Virol. **72**, 9873-80 (1998).

Limited Use License:

Location of features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465
  TATA box: 554–560
  Transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- MCS: 591–671
- Enhanced yellow fluorescent protein (EYFP) gene
  Kozak consensus translation initiation site: 672–682
  Start codon (ATG): 679–681; stop codon: 1396–1398
  Insertion of Val at position 2: 682–684
  GFP-10C mutations (Ser-65 to Gly: 874–876; Val-68 to Leu: 883–885; Ser-72 to Ala: 895–897; Thr-203 to Tyr: 1288–1290)
  His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1552–1557 & 1581–1586
  mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104
  (Packages the noncoding strand of EYFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2166–2171; –10 region: 2189–2194
  Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
  21-bp repeats: 2425–2445, 2446–2466, & 2468–2488
  Early promoter element: 2501–2507
  Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2629–2631; stop codon: 3421–3423
  G→A mutation to remove *Pst* I site: 2811
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

Propagation in *E. coli*:

- Suitable host strains: DH5α, HB101, and other general-purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Gorman, C. (1985) In *DNA cloning: a practical approach, vol. II.* Ed. D. M. Glover. (IRL Press, Oxford, U.K.), pp. 143–190.

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

pET-41a-c(+) Vector

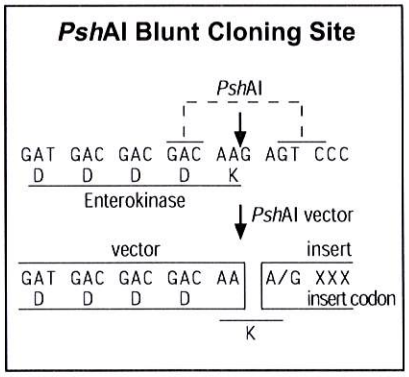
TB239 2/99

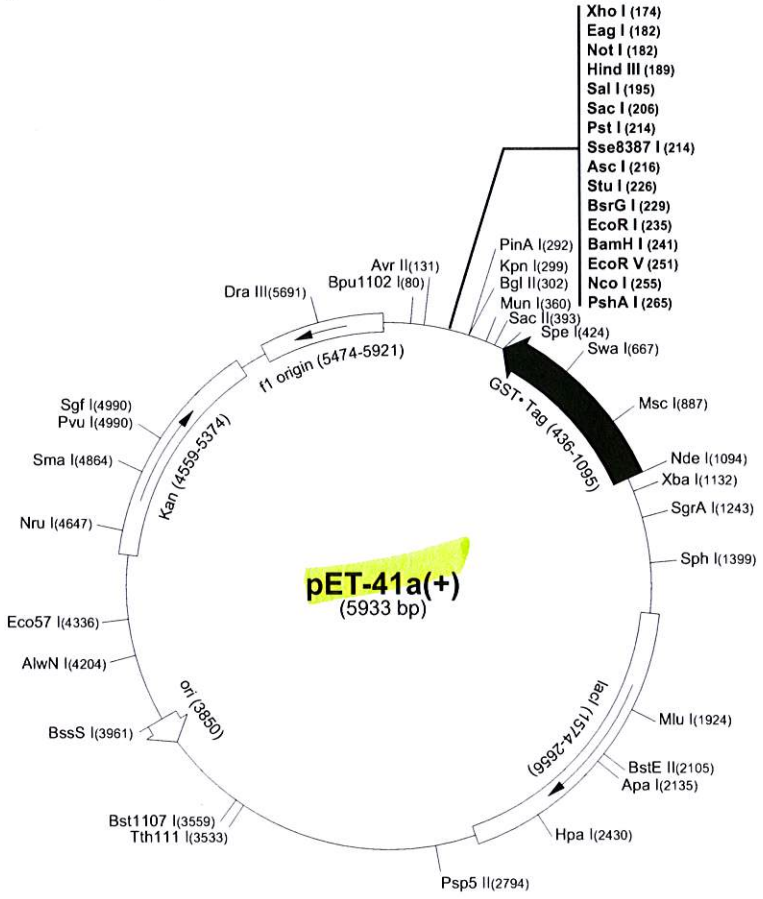
	Cat. No.
pET-41a(+) DNA	70556-3
pET-41b(+) DNA	70557-3
pET-41c(+) DNA	70558-3

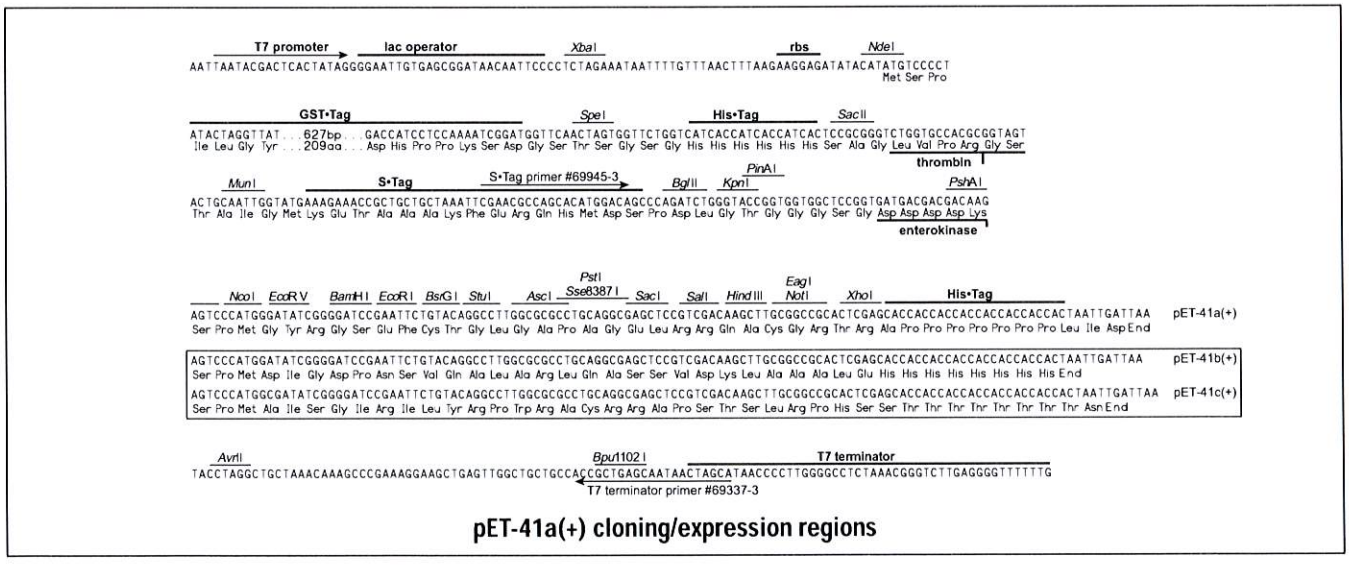
The pET-41 series is designed for cloning and high-level expression of peptide sequences fused with the 220 aa GST•Tag™ protein. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression region of the coding strand transcribed by T7 RNA polymerase is shown below. The f1 origin is oriented so that infection with helper phage will produce virions containing single stranded DNA that corresponds to the coding strand. Therefore, single stranded sequencing should be performed using the T7 terminator primer (cat. no. 69337-3).Vector encoded sequence can be completely removed when cloning into the *Psh*AI site (as shown below) and then cleaving the GST fusion protein with Enterokinase.

pET-41a(+) sequence landmarks

T7 promoter	1167–1183
T7 transcription start	1166
GST•Tag coding sequence	436–1095
His•Tag coding sequence	397–414
S•Tag coding sequence	310–354
Multiple cloning sites (*Psh*AI-*Xho*I)	174–265
His•Tag coding sequence	150–173
T7 terminator	26–72
*lac*I coding sequence	1574–2656
pBR322 origin	3850
Kan coding sequence	4559–5374
F1 origin	5474–5921







pET-41a(+) cloning/expression regions

pET-28a-c(+) Vectors

TB074 12/98

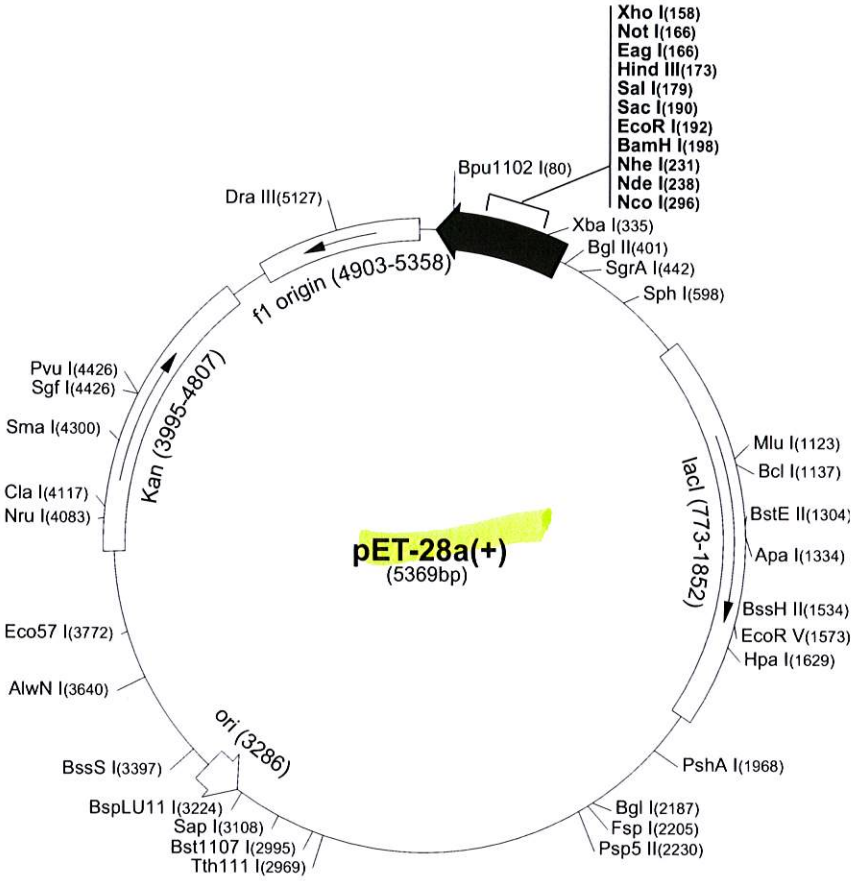
	Cat. No.
pET-28a DNA	69864-3
pET-28b DNA	69865-3
pET-28c DNA	69866-3

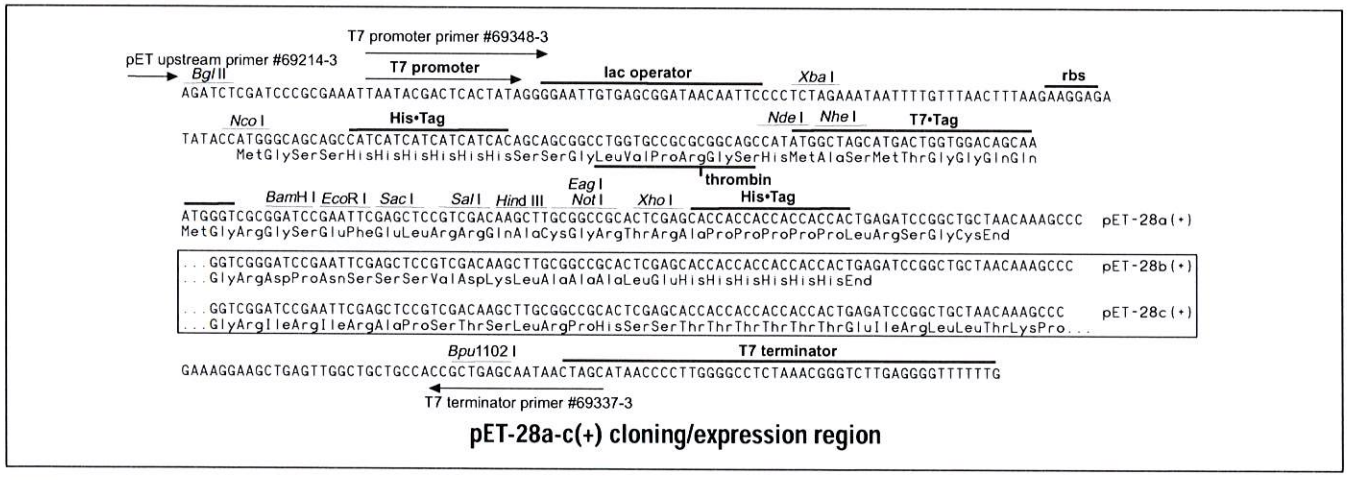
The pET-28a-c(+) vectors carry an N-terminal His•Tag®/thrombin/T7•Tag® configuration plus an optional C-terminal His•Tag sequence. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circular map. The cloning/expression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

pET-28a(+) sequence landmarks

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

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

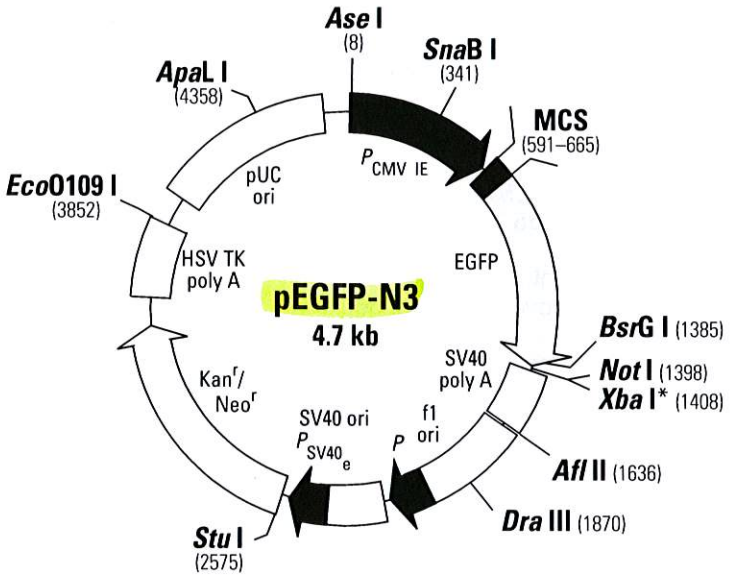


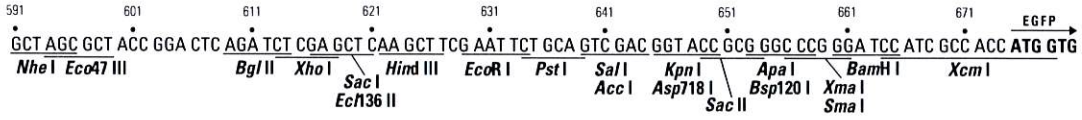


pET-28a-c(+) cloning/expression region

pEGFP-N3 Vector Information PT3054-5

GenBank Accession #: U57609 Catalog #6080-1





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N3 (Unique restriction sites are in bold). The *Not* I site follows the EGFP stop codon. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEGFP-N3 encodes a red-shifted variant of wild-type GFP (1–3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N3 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N3 is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T-antigen. A neomycin resistance cassette (Neor), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli.* The pEGFP-N3 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR29967; published 03 October 2002)

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

Use

Fusions to the C terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-C1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-C1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  - Enhancer region: 59–465; TATA box: 554–560
  - Transcription start point: 583
  - C→G mutation to remove *Sac* I site: 569
- Enhanced green fluorescent protein gene
  - Kozak consensus translation initiation site: 606–616
  - Start codon (ATG): 613–615; Stop codon: 1408–1410
  - Insertion of Val at position 2: 616–618
  - GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  - His-231 to Leu mutation (A→T): 1307
  - Last amino acid in wild-type GFP: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  - Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
  - –35 region: 2164–2169; –10 region: 2187–2192
  - Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  - Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  - 21-bp repeats: 2423–2443, 2444–2464, & 2466–2486
  - Early promoter element: 2499–2505
  - Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  - Neomycin phosphotransferase coding sequences:
  - Start codon (ATG): 2627–2629; stop codon: 3419–3421
  - G→A mutation to remove *Pst* I site: 2809
  - C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  - Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

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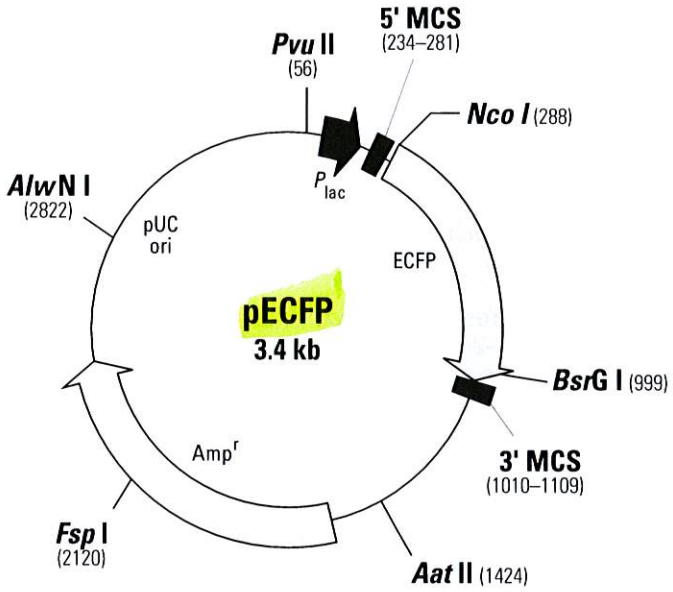
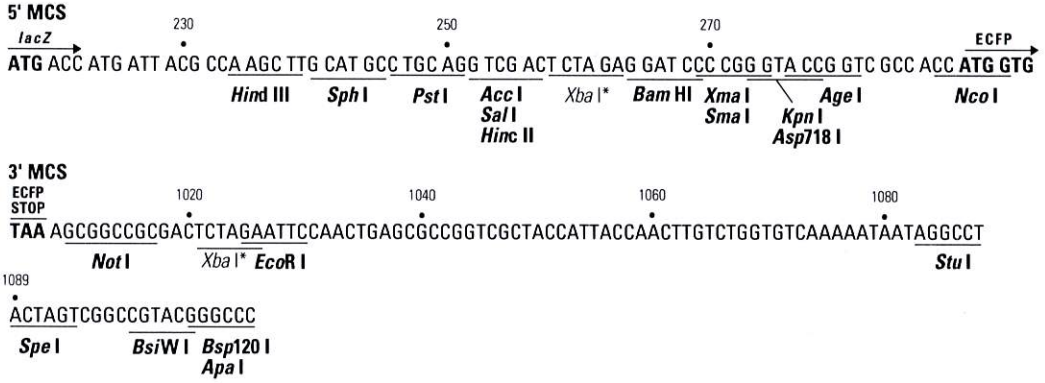
7. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK) pp. 143–190.





Restriction map and multiple cloning site (MCS) of pECFP. Unique restriction sites are in bold. The *Xba* I sites in the 5' and 3' MCSs can be used to excise the ECFP gene.

Description:

pECFP encodes an enhanced cyan fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The ECFP gene contains six amino acid substitutions. The Tyr-66 to Trp substitution gives ECFP fluorescence excitation (major peak at 433 nm and a minor peak at 453 nm) and emission (major peak at 475 nm and a minor peak at 501 nm) similar to other cyan emission variants (1–3). The other five substitutions (Phe-64 to Leu; Ser-65 to Thr; Asn-146 to Ile; Met-153 to Thr; and Val-163 to Ala) enhance the brightness and solubility of the protein, primarily due to improved protein folding properties and efficiency of chromophore formation (2, 4, 5).

In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the ECFP mRNA and consequently the expression of ECFP in mammalian and plant cells.

(PR29963; published 03 October 2002)

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The recombinant EBFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (8). pEBFP-C1 can also be used simply to express EBFP in a cell line of interest (e.g., as a transfection marker).

Location of features:

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
  Enhancer region: 59–465; TATA box: 554–560; transcription start point: 583
  C→G mutation to remove *Sac* I site: 569
- Enhanced blue fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  EBFP mutations (Phe-64 to Leu; Ser-65 to Thr; and Tyr-66 to His): 805–813; Tyr-145 to Phe: 1048–1050
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in EBFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EBFP.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2164–2169; –10 region: 2187–2192
  Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2627–2629; stop codon: 3419–3421
  G→A mutation to remove *Pst* I site: 2809
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

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5. Yang, T. T., *et al.* (1996) *Nucleic Acids Res.* **24**:4592–4593.
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

pcDNA3.1(+)
pcDNA3.1(-)

Catalog nos. V790-20 and V795-20, respectively

Version I
081401
28-0104

Invitrogen™
life technologies

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tech_service@invitrogen.com

Table of Contents

Important Information

Contents

pcDNA3.1 is supplied as follows:

Catalog no.	Contents
V790-20	20 µg pcDNA3.1(+), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0
V795-20	20 µg pcDNA3.1(-), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Product Qualification

Each of the pcDNA3.1 vectors is qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pcDNA3.1(+)	*Nhe* I	5428
	Pst I	1356, 4072
	Sac I	109, 5319
pcDNA3.1(-)	*Nhe* I	5427
	Pst I	1363, 4064
	Sac I	169, 5258
pcDNA3.1/CAT	*Nhe* I	6217
	Pst I	2145, 4072
	Sac I	109, 6008

Methods

Overview

Introduction

pcDNA3.1(+) and pcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (-) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA3.1.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(+)

Below is the multiple cloning site for pcDNA3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(+) is available for downloading from our web site (www.invitrogen.com) or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(+), please refer to the **Appendix**, pages 10-11.

enhancer region (3′ end)

```
689  CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG
                          CAAT                                    TATA
749  TAACAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
          3' end of hCMV
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
     T7 promoter/primer binding site
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
     BamH I                    BstX I*  EcoR I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
      Xba I          Apa I  Pme I
989  AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
          BGH poly (A) site
1109 TCCTTTCCTA ATAAAATGAG GAAATTGCAT
```

putative transcriptional start

Nhe I *Pme* I *Afl* II *Hin*d III *Asp*718 I *Kpn* I

*Eco*R V *Bst*X I* *Not* I *Xho* I

pcDNA3.1/BGH reverse priming site

*Please note that there are two *Bst*X I sites in the polylinker.

continued on next page

Cloning into pcDNA3.1, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F′, DH5α™-T1^R, TOP10) and select transformants on LB plates containing 50 to 100 µg/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 µg aliquots.

Preparing a Glycerol Stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
- Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

Creation of Stable Cell Lines

Introduction

The pcDNA3.1(+) and pcDNA3.1(-) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

Geneticin® Selective Antibiotic

Geneticin® Selective Antibiotic blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn5, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® Selective Antibiotic is available from Invitrogen (Catalog no. 10486-025). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 800 µg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (see below). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take up to 3 weeks of growth in selective media.

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your gene of interest from pcDNA3.1, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. We recommend that you test a range of concentrations to ensure that you determine the minimum concentration necessary for your host cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Geneticin® (0, 50, 100, 200, 400, 600, 800 µg/ml Geneticin®).
3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Geneticin® that prevents growth within 2-3 weeks after addition of Geneticin®.

continued on next page

Creation of Stable Cell Lines, continued

Selection of Stable Integrants

Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

1. Transfect your mammalian host cell line with your pcDNA3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA3.1/CAT plasmid as a positive control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
4. Feed the cells with selective medium every 3-4 days until Geneticin®-resistant foci can be identified.
5. Pick and expand colonies in 96- or 48-well plates.

pcDNA3.1 Vectors, continued

Features of pcDNA3.1(+) and pcDNA3.1(-)

pcDNA3.1(+) (5428 bp) and pcDNA3.1(-) (5427 bp) contain the following elements. All features have been functionally tested.

Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in forward or reverse orientation	Allows insertion of your gene and facilitates cloning
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*

Technical Service

World Wide Web

Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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2. Follow instructions on the page and fill out all the required fields.
3. To request additional MSDSs, click the 'Add Another' button.
4. All requests will be faxed unless another method is selected.
5. When you are finished entering information, click the 'Submit' button. Your MSDS will be sent within 24 hours.

continued on next page

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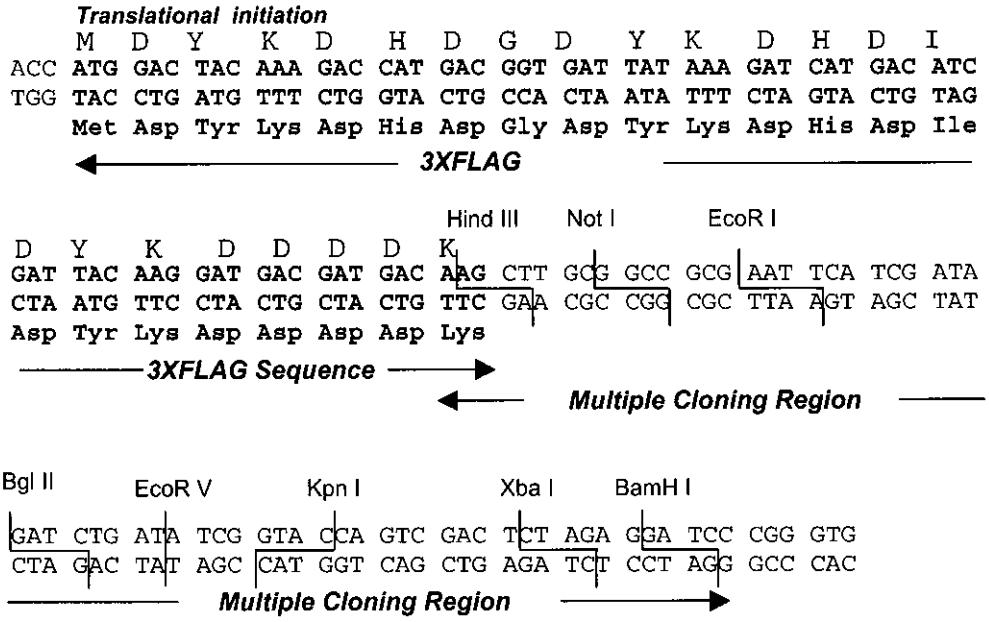
Nucleotide Sequence of the Multiple Cloning Region of the p3XFLAG-CMV-10 Expression Vector

Sequence range: 925 to 1061



07/03

Public Health Agency of Canada
Centre for Emergency Preparedness and Response

Agence de la santé publique du Canada
Centre de mesures et d'interventions d'urgence

Permit no.-Permis no.

Permit to import human pathogen(s)

Permis d'importation d'agent(s) anthropopathogène(s)

P- 17116

Under the authority of the Human Pathogens Importation Regulations.

Sous le régime du Règlement sur l'importation des agents anthropopathogènes.

Importer-Name, address and postal code - **Importateur**-Nom, adresse et code postal

Facsimile-Télécopieur

Telephone no.- No. de téléphone

University of Western Ontario
Department of Microbiology and Immunology
1151 Richmond Street
London, ON N6A 5C1

519-661-3499

519-661-3438

Attn: Dr. Stephen Barr

Supplier-Name and address - **Fournisseur**-Nom et adresse

NIH Nonhuman Primate Regent Resource
Beth Israel Deaconess Medical Center
Division of Viral Pathogenesis, E/CLS 1038
330 Brookline Avenue
Boston, MA 02215, USA

Name(s) of Port(s) of Entry- To Clear Customs at Port(s) of entry
Nom(s) de(s) point(s) d'entrée -Dédouanement au(x) point(s) d'entrée

Various ports

Description of Pathogen(s)-For the importation of- **Description de(s) agent(s) anthropopathogène(s)**-Pour l'importation

Cynomolgus T cell line(HSC-F) from Macaca fascicularis immortalized by transformation with Herpes virus saimiri*.

***Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for importation -**
***Les agents anthropopathogènes indiqués sur ce permis doivent aussi être accompagnés d'un permis d'importation de l'ACIA.**

On the following terms and conditions as marked:-Selon les conditions indiquées:

1.	Work involving any of the imported material shall be limited to *in vitro* laboratory studies.	■ Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire *in vitro*.
2.	Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.	■ Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.
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5.	Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.	■ Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.
6.	No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	■ La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis. AUCUNE AGENT ANTHROPOPATHOGÈNE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
7.	On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.	■ Au terme des travaux de l'importateur auxquels a servi l'agent anthropopathogène importé, celui-ci et tous ses dérivés doivent être détruits.
8.	Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.	■ On peut accomplir l'isolation, l'identification primaire, et/ou la manipulation au niveau de confinement 2 (exigences physiques) en utilisant les exigences opérationnelles de niveau de confinement 3.
9.	NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	☐ AUCUNE MATIÈRE IMPORTÉE NE PEUT ÊTRE TRANSPORTÉE, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MISE EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
10.	The Director must approve all new work with the imported material involving construction of recombinants that requires an increase of containment from level 2.	☐ Tous nouveaux travaux de manipulation génétique (recombiné) avec la matière importée qui demandera que le niveau 2 de confinement soit augmenté exigera l'approbation du Directeur.
11.	No culturing of Risk Group 3 or 4 pathogens shall be done.	■ Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise.

12. **This permit is valid only for:** / **Le présent permis n'est valide que pour:**

☐ a) a single entry into Canada **or** une seule entrée au Canada **ou**

■ b) importations at intervals of during the period beginning on and ending on

immortalized by transformation with Herpes virus saimiri*.

***Pathogen(s) indicated on this permit also require an accompanying valid CFIA permit for importation -**
***Les agents anthropopathogènes indiqués sur ce permis doivent aussi être accompagnés d'un permis d'importation de l'ACIA.**

On the following terms and conditions as marked:-Selon les conditions indiquées:

1.	Work involving any of the imported material shall be limited to *in vitro* laboratory studies.	■ Les travaux auxquels la matière importée est destinée doivent se limiter à des études de laboratoire *in vitro*.
2.	Domestic animals, including poultry, cattle, sheep, swine and horses, shall not be directly or indirectly exposed to infection by any of the imported material.	■ Les animaux domestiques, y compris les volailles, bovins, ovins, porcins et chevaux, ne doivent pas être exposés, directement ou indirectement, à l'infection par la matière importée.
3.	All animals exposed to infection by any of the imported material shall be so exposed and held only in isolated insect-and rodent-proof facilities.	☐ Les animaux exposés à l'infection par la matière importée doivent y être exposés et être gardés uniquement dans des installations isolées à l'abri des insectes et des rongeurs.
4.	All equipment, animal pens, cages, bedding, waste and other articles under the importer's control, that come in direct or indirect contact with any of the imported material, shall be sterilized by autoclaving or incinerated.	■ L'équipement, les enclos pour animaux, les cages, les litières, les déchets et tout autre article sous la responsabilité de l'importateur qui viennent en contact direct ou indirect avec la matière importée doivent être stérilisés par autoclavage ou incinérés.
5.	Packaging materials, containers and all unused portions of the imported material shall be sterilized by autoclaving or incinerated.	■ Le matériel d'emballage, les récipients et toute partie inutilisée de la matière importée doivent être stérilisés par autoclavage ou incinérés.
6.	No work on the imported material shall be done, except work conducted or directed by the importer in the facilities described in the application for this permit. NO HUMAN PATHOGEN BELONGING TO RISK GROUP 3 OR 4 MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	■ La matière importée ne peut servir qu'aux travaux effectués ou dirigés par l'importateur dans les installations décrites dans la demande de permis. AUCUNE AGENT ANTHROPOPATHOGÈNE DU GROUPE DE RISQUE 3 OU 4 NE PEUT ÊTRE TRANSPORTÉ, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MIS EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
7.	On completion of the importer's work involving the imported human pathogen, the pathogen and all its derivatives shall be destroyed.	■ Au terme des travaux de l'importateur auxquels a servi l'agent anthropopathogène importé, celui-ci et tous ses dérivés doivent être détruits.
8.	Primary isolation, identification and/or manipulation may be done in level 2 containment (physical requirements) using containment level 3 operational requirements.	■ On peut accomplir l'isolation, l'identification primaire, et/ou la manipulation au niveau de confinement 2 (exigences physiques) en utilisant les exigences opérationnelles de niveau de confinement 3.
9.	NO IMPORTED MATERIAL MAY BE REMOVED TO ANOTHER LOCATION, OR TRANSFERRED INTO THE POSSESSION OF A PERSON OTHER THAN THE IMPORTER, WITHOUT THE PERMISSION OF THE DIRECTOR.	☐ AUCUNE MATIÈRE IMPORTÉE NE PEUT ÊTRE TRANSPORTÉE, SANS LA PERMISSION DU DIRECTEUR, VERS UN AUTRE LIEU OU ÊTRE MISE EN LA POSSESSION D'UNE AUTRE PERSONNE QUE L'IMPORTATEUR.
10.	The Director must approve all new work with the imported material involving construction of recombinants that requires an increase of containment from level 2.	☐ Tous nouveaux travaux de manipulation génétique (recombiné) avec la matière importée qui demandera que le niveau 2 de confinement soit augmenté exigera l'approbation du Directeur.
11.	No culturing of Risk Group 3 or 4 pathogens shall be done.	■ Aucune culture d'agent anthropopathogène du Groupe de risque 3 ou 4 ne sera entreprise.

12. **This permit is valid only for:** ☐ a) a single entry into Canada **or**
Le présent permis n'est valide que pour: une seule entrée au Canada **ou**

■ b) importations at intervals of — les importations effectuées à intervalles de — during the period beginning on — au cours de la période commençant le — AUGUST 17, 2009 — and ending on — et se terminant le — AUGUST 31, 2010

Authorization-Signature of Director
Autorisation-Signature du Directeur

Marianne Heisz for

Date AUGUST 17, 2009

Note: Transporting and otherwise dealing with imported material are subject to federal, provincial and municipal laws (if any), to the extent that, those laws apply in respect of that material.

Nota : Les opérations relatives à la matière importée, y compris le transport, sont assujetties aux lois fédérales, provinciales et aux règlements municipaux applicables.

Canada

Canadian Food Inspection Agency
Goverment of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
A-2009-03559-4
ORIGINAL
2009/08/04
year/mo/day
année/mois/jour

IMPORT PERMIT PERMIS D'IMPORTATION

THIS PERMIT IS ISSUED PURSUANT TO:/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A:

THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur	**Exporter/Exportateur**
UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE DEPART. OF MICROBIOLOGY AND IMMUNOLOGY DENTAL SCIENCES BUILDING LONDON, ONTARIO N6A5C1 Applicant Name: DR. STEPHEN BARR Phone: 519-661-3438 Fax: 519-661-3499 Email: STEPHEN.BARR@BARR@UWO.CA	NJH NONHUMAN PRIMATE REAGENT RESOURCES 330 BROOKLINE AVENUE BOSTON, MASSACHUSETTS UNITED STATES 02215 Contact: Keith A. Reimann Phone: 617-735-4476 Fax: 617-735-4527
Quarantine/Destination/Quarantaine	**Producer/Producteur**
Valid/Valide from/du 2009/08/04 year/month/day année/mois/jour **to/au** 2010/08/31 year/month/day année/mois/jour	**Country of Origin/ Pays d'Origine** UNITED STATES (MASSACHUSETTS)

For the entry of/ Pour l'entrée de: Single shipment/Chargement simple XX Multiple shipments/Chargements multiples

Place of entry into Canada/Lieu d'entrée au Canada:

FOR THE IMPORTATION OF:/POUR L'IMPORTATION DE:
(Description of things(s)/Description de la ou des choses)
1. Product Description: CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRSNFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI.

(TO BE USED IN VITRO ONLY IN DENTAL SCIENCES BLDG ROOM 6006, DEPT. OF MICROBIOLOGY & IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON) Proposed End Use: "In Vitro" Scientific Name: Biocontainment Level: 2

A PERSON WHO IMPORTS A THING UNDER THIS PERMIT SHALL COMPLY WITH ALL THE CONDITIONS SET OUT HEREIN/TOUTE PERSONNE QUI IMPORTE UNE CHOSE EN VERTU DE CE PERMIS DEVRA RESPECTER TOUTES LES CONDITIONS DÉCRITES CI-DESSOUS

Selected Conditions / Conditions Choisies

CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRSNFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI.

(TO BE USED IN VITRO ONLY IN DENTAL SCIENCES BLDG ROOM 6006, DEPT. OF MICROBIOLOGY & IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON)

1. The original or a copy of the signed original of this permit and any other necessary import / export documentation pertaining to the shipment of animal(s) or thing(s) must be provided for inspection at the first port of entry or to a Canadian Food Inspection Agency Import Service Center.

2. The conditions in this permit can only be changed or amended by a CFIA inspector. Any change to the permit by an unauthorized person will render the permit invalid.

Canadian Food Inspection Agency
Government of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

Permit No./N° de permis:
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IMPORT PERMIT PERMIS D'IMPORTATION

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THE HEALTH OF ANIMALS ACT AND REGULATIONS/LOI ET RÈGLEMENT SUR LA SANTÉ DES ANIMAUX

Importer/Importateur	Exporter/Exportateur
UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE DEPART. OF MICROBIOLOGY AND IMMUNOLOGY DENTAL SCIENCES BUILDING LONDON, ONTARIO N6A5C1 Applicant Name: DR. STEPHEN BARR Phone: 519-661-3438 Fax: 519-661-3499 Email: STEPHEN.BARR@BARR@UWO.CA	NIH NONHUMAN PRIMATE REAGENT RESOURCES 330 BROOKLINE AVENUE BOSTON, MASSACHUSETTS UNITED STATES 02215 Contact: Keith A. Reimann Phone: 617-735-4476 Fax: 617-735-4527

Selected Conditions / Conditions Choisies (Continued/Suite)

3. The imported material must be packaged in appropriate shipping containers to prevent accidental spillage of contents during shipping. Importers should be aware of their obligations under Transport Canada's regulations concerning transportation of dangerous goods.

4. All infectious material must be handled in appropriate animal pathogen containment level 2 facilities as described in Containment Standards for Veterinary Facilities, 1996, AAFC publication no. 1921.

5. The material authorized for importation by this permit is to be used in in vitro studies ONLY and must not to be introduced into laboratory, domestic or wild animals (including birds or fish) unless written authorization is obtained from the Canadian Food Inspection Agency.

6. The animal(s) or thing(s) imported under this permit must NEVER be removed from the premises of destination listed on this permit, even after the animals have been released from their post-import quarantine, unless written authorization is obtained from the Canadian Food Inspection Agency.

7. Upon completion of the tests or experiments, the imported material as described on this permit and any derivatives thereof must be autoclaved, incinerated or alternatively disposed of in a manner approved by an inspector of the Canadian Food Inspection Agency.

8. Records pertaining to the imported product's use, storage and disposal must be maintained for two (2) years following importation. These records must be made available for inspection by the Canadian Food Inspection Agency upon request.

9. The importer is responsible for all costs incurred or associated with any testing or treatment of the animal(s) or thing(s) that may be required under the import permit or under the authority of the Health of Animals Act or the Health of Animals Regulations. The importer shall pay all fees for services required in respect of the importation under the National Animal Health Program Cost Recovery Fees Regulations in place at the time of importation.

10. Consideration of an application necessary for issuance of a permit to import the described animal or thing is subject to Class I fees.

11. The issuance of this permit does not relieve the owner or the importer of the obligation to comply with any other relevant federal, provincial or municipal legislation or requirement.

12. Failure to comply with the conditions contained in this permit or with the provisions of the Health of Animals Act and Regulations may result in the cancellation of this permit and will result in the forfeiture to the Crown of the imported thing(s) or in the removal of the thing(s) from Canada, all without compensation to, and at the expense of the importer. The importer(s) are responsible for the imported thing(s), their freedom from extraneous disease, active or latent, and genetic or other defects. The importer, his heirs, executors, successors and assigns release and discharges Her Majesty the Queen in right of Canada and the CFIA of and from all claims and demands, damages, actions or causes of action arising or to arise by reason of the importation of the thing(s) and agrees to indemnify and save harmless Her Majesty the Queen in right of Canada and the CFIA from and against all actions, damages, claims and demands which may be brought in respect of or arising out of the importation of such thing(s), any contamination with extraneous disease or other defects.

Canadian Food Inspection Agency
Goverment of Canada

Agence canadienne d'inspection des aliments
Gouvernement du Canada

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IMPORT PERMIT PERMIS D'IMPORTATION

THIS PERMIT IS ISSUED PURSUANT TO:/CE PERMIS EST DÉLIVRÉ CONFORMÉMENT A:

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Importer/Importateur	Exporter/Exportateur
UNIVERSITY OF WESTERN ONTARIO - ANIMAL CARE DEPART. OF MICROBIOLOGY AND IMMUNOLOGY DENTAL SCIENCES BUILDING LONDON, ONTARIO N6A5C1 Applicant Name: DR. STEPHEN BARR Phone: 519-661-3438 Fax: 519-661-3499 Email: STEPHEN.BARR@BARR@UWO.CA	NIH NONHUMAN PRIMATE REAGENT RESOURCES 330 BROOKLINE AVENUE BOSTON, MASSACHUSETTS UNITED STATES 02215 Contact: Keith A. Reimann Phone: 617-735-4476 Fax: 617-735-4527

Selected Conditions / Conditions Choisies (Continued/Suite)

13. This permit is conditional upon a permit being obtained under the Human Pathogens Importation Regulations to import the pathogenic material and upon that import permit being produced and valid when the above pathogenic material is presented to an inspector for inspection at the time of importation.

Additional Conditions Additionnelles

CYNOMOLGUS T CELL LINE (HSC-F) FROM MACACA FASCICULARIS: IMMORTALIZED CELL LINE DERIVED BY TRSNFORMATION OF CYNOMOLGUS MONKEY FETAL SPLENOCYTES WITH HERPESVIRUS SAIMIRI.

(TO BE USED IN VITRO ONLY IN DENTAL SCIENCES BLDG ROOM 6006, DEPT. OF MICROBIOLOGY & IMMUNOLOGY, UNIVERSITY OF WESTERN ONTARIO, LONDON, ON)

1. LEVEL 2 PHYSICAL CONTAINMENT AND LEVEL 3 OPERATIONAL PRACTICES ARE REQUIRED.
2. NO CULTURING OF CONTAINMENT LEVEL 3 OR 4 PATHOGENS SHALL BE DONE.

Cinthia Labrie

Authorized By:/Approuvé par:
CINTHIA LABRIE

For the Minister of Agriculture and Agri-Food
Pour le ministre d'agriculture et agroalimentaire

The information is required by (for) the Canadian Food Inspection Agency for the purpose of verifying import products. Information may be accessible or protected as required under the provisions of the Access to Information Act.

Canada