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

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

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

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

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

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

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

PRINCIPAL INVESTIGATOR:	**Jimmy Dikeakos**
DEPARTMENT:	**Microbiology & Immunology**
ADDRESS:	**Siebens-Drake Research Institute**
PHONE NUMBER:	**x82302**
EMERGENCY PHONE NUMBER(S):	**519-639-5421**
EMAIL:	**ddikeako@uwo.ca**

Location of experimental work to be carried out :

Building : **Siebens-Drake Research Institute** Room(s): **237**

Building : **Siebens-Drake Research Institute** Room(s): **238**

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): **To be Submitted: 1- CIHR-Role of the Nef-SFK axis in HIV-1 pathogenesis**

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

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.

Experiments with Bacteria:
a. Plasmid preparations
Strains that will be used for cloning purposes (Escherichia coli DH5alpha) will be used to replicate DNA plasmids for subsequent purification. Culture flasks are decontaminated in 10% bleach and subsequently washed with a detergent based soap diluted in water and autoclaved.

b. Protein expression
Strains that will be used to express proteins (Escherichia coli BL21 (DE3) pLys) will be used to express recombinant proteins in large amounts for subsequent purification. Culture flasks are decontaminated in 10% bleach and subsequently washed with a detegent based soap diluted in water and autoclaved.

Experiments with Mammalian Material:
a. Cell lines
We will culture numerous cell lines in our Level 2+ room (SDRI; Room 238). Cells will be maitained in a 37 degrees 5% CO2 incubator and sub-cultured 3 times a week to ensure propagation. Cells will be transfected using various techniques including cationic lipid DNA transfer, nucleoporation and calcium phosphate transfections. All of the above mammalian cell experiments will be performed in a biological safety cabinet. All equipment in contact with the mammalian cells will be decontaminated with 10% bleach and washed with a detergent based soap diluted in water and autoclaved.
b. Pseudotype Human Immunodefficiency Virus-1 (HIV-1)
Replication inocompetent HIV-1 vectors will be used to introduce genes that are not known to be growth-altering into human cells for stable expression resulting into gene intergration in the cells. Pseudotyped virus is generated by transfecting three plasmids in the human 293T cell line. The three plasmids are as follow: 1. DeltaR8.2: the packaging plasmid containing genes to produce the structure of the virus. This plasmid lacks essential elements required for replication including long-terminal-repeat (LTR) regions , the genome packaging signal, the envelopple (env) gene and the vpu gene. 2. VSVg , codes for an essential enveloppe protein from (Vesicular Stomatitis Virus) allowing cell entry. This plasmid is provided in trans and will not be packaged in the viruses's new genome. The role of this plasmid is to provide the enveloppe protein essential for viral entry into the target cell. 3. Ppt contains two LTRs and a multiple cloning site in order to insert the gene of choice. This plasmid serves to deliver the gene of choice and is under the control of a CMV promoter. This is the gene that will be integrated into the target cell genome. The resulting virus will be replication incompetent and work will be performed with SDRI 238 under Biosafety 2+3 conditions and enhanced safety precautions. All equipments/supplies in contact with the virus will be decontaminated with 10% bleach and subsequently washed with a detergent based soap diluted in water and will be autoclaved.

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

1. The AIDS pandemic has resulted in over 25 million deaths worldwide and there are almost 3 million new HIV-1 infections each year. Currently, the majority of treatment regimens rely on anti-retroviral therapies that target the few HIV-1 proteins that possess intrinsic enzyme activity. Unfortunately, the high mutation rate and immune evasive capabilities of HIV-1 diminish the therapeutic effectiveness of these strategies and underscore the need for innovative approaches that will identify novel therapeutic targets .

The HIV-1 protein Nef, which is required for the onset of AIDS following HIV-1 infection, has long been overlooked as a potential therapeutic target because it lacks intrinsic enzyme activity. Nef reprograms infected cells to support HIV-1 replication and escape immune surveillance by assembling a multi-kinase complex consisting of multiple cellular proteins, including Src Family Kinases (SFKs) and the membrane trafficking proteins PACS-1 and PACS-2. Indeed, studies in transgenic mice demonstrated Nef must bind SFKs to cause an AIDS-like disease. Thus, although Nef lacks intrinsic enzymatic activity, it recruits host cell enzymes to drive disease. Taken together, these findings suggest that Nef may be a novel target for the development of HIV-1 therapeutics.

Recently, we determined that the small chemical probe 2c selectively interferes with the binding of Nef to SFKs and blocks Nef-mediated immune evasion, providing a proof-of-concept that Nef may be a target for novel HIV-1 therapeutics. However, the narrow therapeutic window of 2c precludes its development as a potential drug. Therefore, we conducted a small-scale computer-based virtual chemical screen to identify drug-like compounds that may inhibit the Nef-SFK interaction. This initial screen yielded two compounds that block Nef-mediated signaling complex formation and HIV-1 replication, thereby demonstrating feasibility of a large-scale virtual drug screen to identify novel HIV-1 therapeutics.

In this proposal, we will identify novel drug-like compounds that block the Nef-SFK interaction by performing a large-scale virtual screen of a diverse Proctor & Gamble small molecule library that was desigend to include only drug-like molecules. Our preliminary data demonstrate the feasibility of identifying drug-like Nef-SFK inhibitors from this library that are capable of disrupting Nef-dependent pathways. Using these initially identified molecules as templates, we expect to identify highly potent drug-like molecules that inhibit the Nef-SFK interaction and therefore block the ability of Nef to drive HIV-1 disease.

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 Immunodefficiency Pseudovirus	☒ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.1**	**NIH AIDS Reagents Programme**	☐ 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	**4**	**Agilent Technologies**	☒ 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
	☐ 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		
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	**Please see following page**		
Rodent	☐ Yes ☒ No			
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	**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	HeLa	2	Human Papilloma Virus	ATCC
	Yes	Jurkat E6-1	1		ATCC
	Yes	CEM-SS	1		NIH AIDS Reagents
	Yes	H9	1		NIH AIDS Reagents

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)	**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 program**	**Nef, Vpu, shRNA, scrambled, eGFP**	**Cells express tranduced genes resulting in overexpression 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 **Nef, Gag, Pol, Env, Tat,Rev**
- 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	Nef, PACS1 and PACS2	None known	None	Plasmid propagation
pLKO.1	Open Biosystems	shRNA (scrambled)	None known	None	Plasmid propagation
pDR8.2	Didier Trono (Addgene)	HIV genomve minus envelopp	None known	None	Plasmid propagation
pVSVG	Didier Trono (Addgene)	Vesicular stomatitis virus protein G	None known	None	Plasmid propagation
pPPT	Didier Trono (Addgene)	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
pGEX 4T.1	GE Healthcare	Nef, PACS1 , PACS2	None known	None	Plasmid propagation
pET41a	Novagen	Nef, PACS1 , PACS2	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 yet submitted to committee**

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** ☐ NO
If **NO**, please proceed to Section 13.0

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

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

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

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Jimmy Dikeakos ***Date:*** July 25, 2012

14.0 Containment Levels

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

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

14.3 Please indicate permit number (not applicable for first time applicants):

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 areas (s) will be scrubbed with soapy water and liberally rinsed with water. Victim will be directed to Staff Health during business hours or to the local 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 **Jimmy Dikeakos** ***Date:*** **July 25, 2012**

15.4 Additional Comments: **For section 12.4 ; a permit will not be required because there will be no Level 2 material that will be imported.**

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:

ATTACHMENTS

ATCC

MATERIAL SAFETY DATA SHEET

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

MATERIAL SAFETY DATA SHEET

SECTION 1 - SUBSTANCE IDENTITY AND COMPANY INFORMATION

Product Name: Various Animal Cell Cultures at Biosafety Level 1 or 2
ATCC Catalog #: Various

COMPANY INFORMATION: AMERICAN TYPE CULTURE COLLECTION
PO BOX 1549
MANASSAS, VA 20108

FOR INFORMATION CALL: 800-638-6597 or 703-365-2700
AFTER-HOURS CONTACT: 703-365-2710
CHEMTREC EMERGENCY: 800-424-9300 or 703-527-3887

SECTION 2 - COMPOSITION/INFORMATION ON INGREDIENTS

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). Frozen Cultures may also contain a 5%-10% solution of Dimethyl sulfoxide as a cryoprotectant.

SECTION 3 - HAZARD IDENTIFICATION

HMIS Rating:	Health: 0	Flammability: 0	Reactivity:0
NFPA Rating:	Health:0	Flammability:0	Reactivity:0

This substance is not hazardous as defined by OSHA 29CFR 1910.1200 however this product should be handled according to good lab practices, with proper personal protective equipment, proper engineering controls and within the parameters of the purchaser's safety program.

Health Hazards

For Biosafety Level 1 Cell Cultures
Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.
This cell line is not known to cause disease in healthy adult humans. These cells have **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

See next page for Biosafety Level 2 cell cultures.

For Biosafety Level 2 Cell Cultures
Handle as a potentially biohazardous material under at least Biosafety Level 2 containment.
These cell lines are associated with human disease, hazards include: percutaneous injury, ingestion, mucous membrane exposure (U.S. Government Publication **Biosafety in Microbiological and Biomedical Laboratories)**. These cells have **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents, unless otherwise reported on the Certificate of Analysis. Regardless of results reported on the Certificate of Analysis Universal Precautions according to 29 CFR 1910.1030 should be followed at all times when manipulating these cell lines.

SECTION 4 - FIRST AID MEASURES

Report to your Safety Office and Seek Medical Attention as Soon as Possible

Ingestion: If person is unconscious seek emergency medical attention; never give anything by mouth to an unconscious person. If the person is conscious wash mouth out with copious amounts of water and call a physician then administer three cupfuls of water. Do not induce vomiting unless directed to do so by a physician.

Inhalation: If person is unconscious seek emergency medical attention, if person is conscious remove to fresh air and call a physician.

Dermal exposure: Immediately wash skin with copious amounts of water followed by washing with soap and copious amounts of water. Remove all contaminated clothing.

Eye exposures: Flush eyes with copious amounts of water for at least 15 minutes with eyelids separated and call a physician.

SECTION 5 - FIRE FIGHTING MEASURES

Flammability: Data not available

Suitable Extinguishing Media: Water spray, carbon dioxide, dry chemical powder, Halon (where regulations permit), or appropriate foam.

Protective Equipment: Wear self-contained breathing apparatus and protective clothing to prevent inhalation, ingestion, skin and eye contact.

Specific Hazard(s): Responders should take into consideration the biohazard risk associated with responding to a fire in the area where the material may be stored or handled.

SECTION 6 - ACCIDENTAL RELEASE MEASURES

Procedure(s) of Personal Precaution(s): At a minimum use PPE listed in Section 8. Wear laboratory coat, gloves and eye protection. Avoid all contact.

Methods for Cleaning Up

Patient/Victim: Wash with soap and water. Work clothes should be laundered separately. Launder contaminated clothing before re-use. Do not take clothing home.
Equipment/Environment: Allow aerosols to settle; wearing protective clothing, gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time before clean up (30 min).
Note: The use of additional PPE may be necessary for cleaning solutions.

SECTION 7 - HANDLING AND STORAGE

Handle and store according to instructions on product information sheet and label.
Special Requirements:
Follow established laboratory procedures when handling material.

SECTION 8 - EXPOSURE CONTROLS/PERSONAL PROTECTION

Use Personal Protective Equipment: Including Eye Protection, Chemical Resistant Gloves, and appropriate clothing to prevent skin exposure. In addition, a Respiratory protection program that complies with OSHA 29 CFR 1910.134 and ANSI Z88.2 requirements or European Standard EN 149 must be followed whenever workplace conditions warrant respirator use.

Engineering Controls: The use and storage of this material requires user to maintain and make available appropriate eyewash and safety shower facilities. Use fume hood or other appropriate ventilation method to keep airborne concentrations a low as possible.

Exposure Limits: No exposure limits for this material have been established by ACGIH, NIOSH, or OSHA.

SECTION 9 - PHYSICAL AND CHEMICAL PROPERTIES

Data Not Available

SECTION 10 - STABILITY AND REACTIVITY

Hazardous polymerization will not occur.

SECTION 11 - TOXICOLOGICAL INFORMATION

Route of Exposure

Eye Contact: Data not available. Avoid eye contact.
Skin Contact: Data not available. Avoid skin contact.
Skin Absorption: Data not available. Avoid skin absorption.
Inhalation: Data not available. Avoid inhalation.
Ingestion: Data not available. Avoid ingestion.
Parenteral Exposure: Data not available. Avoid parenteral exposure.

Sensitization
Skin: Data not available
Respiratory: Data not available

Target Organ(s) or System(s): Data not available

Signs and Symptoms of Exposure
Skin and Mucous Membranes: Data not available
Respiratory: Data not available
Gastrointestinal: Data not available

Toxicity Data: Data not available
Effects of Long Term or Repeated Exposure: Data not available
Chronic Exposure–Teratogen: Data not available
Chronic Exposure–Mutagen: Data not available
Chronic Exposure–Reproductive Hazard: Data not available

SECTION 12 - ECOLOGICAL INFORMATION

No ecological information available.

SECTION 13 - DISPOSAL CONSIDERATIONS

Decontaminate all wastes before disposal (steam sterilization, chemical disinfection, and/or incineration).

Dispose of in accordance with applicable regulations.

SECTION 14 - TRANSPORT INFORMATION

Contact ATCC for transport information.

SECTION 15 - REGULATORY INFORMATION

Contact ATCC for regulatory information.

SECTION 16 - OTHER INFORMATION

THE INFORMATION PRESENTED IN THIS DOCUMENT IS BELIEVED TO BE CORRECT BASED UPON DATA AVAILABLE TO ATCC. USERS SHOULD MAKE AN INDEPENDENT DECISION REGARDING THE ACCURACY OF THIS INFORMATION BASED ON THEIR NEEDS AND DATA AVAILABLE TO THEM. ALL SUBSTANCES AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND ALL NECESSARY SAFETY PRECAUTIONS SHOULD BE TAKEN. ATCC ASSUMES NO LIABILITY RESULTING FROM USING OR COMING IN CONTACT WITH THIS SUBSTANCE.

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HUMAN IMMUNODEFICIENCY VIRUS

PATHOGEN SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: Human immunodeficiency virus (HIV).

SYNONYM OR CROSS REFERENCE: HIV, acquired immune deficiency syndrome, AIDS [1-20]. Was previously known as lymphadenopathy-associated virus, human T-lymphotropic virus type III (HTLV-III), immunodeficiency-associated virus, and AIDS-associated retrovirus [1-20].

CHARACTERISTICS: HIV is a member of the *Retroviridae* family, genus *Lentivirus* [14, 16]. HIV is an icosahedral, enveloped virus, of approximately 100 to 110 nm in diameter, and has a single-stranded, linear, positive-sense RNA genome [14, 16]. HIV has two recognised strains: HIV-1 and HIV-2 [11, 16, 17]. Upon entry into the host cell, retroviral RNA is converted to DNA by a virally encoded reverse transcriptase enzyme, the DNA transcript is integrated into the host's chromosomal DNA [14].

SECTION II - HAZARD IDENTIFICATION

PATHOGENICITY/TOXICITY: AIDS is characterised by symptoms and infections caused by the breakdown of the immune system (by destruction or functional impairment of CD4 receptors) due to HIV infection [10, 12]. HIV can infect many cell types, mainly lymphocytes, but also macrophages, and microglia in the brain, and other neurological cells, resulting in profound asthenia, dementia and damage to the peripheral nervous system [12]. Due to immunodeficiency, patients succumb to various fungi, parasites, bacteria, and/or viruses and are prone to certain tumours [10, 12]. Globally, *Mycobacterium tuberculosis* is the most common cause of death of HIV-infected individuals. The clinical features of HIV infection vary depending on the stage of the disease [6]. Acute infection is accompanied by non-specific "flu-like" and "mononucleosis-like" symptoms such as myalgia, arthralgia, diarrhoea, nausea, vomiting, headache, hepatosplenomegaly, weight loss, and neurological symptoms [6, 16, 21]. Early-stage disease refers to the period of clinical latency between the time of the primary infection and the development of symptoms indicative of advanced immunodeficiency. Typically, when the patient's CD4+ T-cell count falls below 500 cells/µL, syndromes indicative of depressed cell mediated immunity can appear. Examples include oropharyngeal and recurrent vulvovaginal candidiasis, bacillary angiomatosis, recurrent or multidermatomal herpes zoster, listeriosis, infections due to *Rhodococcus equi*, pelvic inflammatory disease, oral hairy leukoplakia associated with Epstein-Barr virus, cervical dysplasia, long lasting diarrhoea, idiopathic thrombocytopenic purpura, and peripheral neuropathy [21]. Late-stage disease refers to the period when the patient's CD4+ T-cell count falls below 200 cells/µL [10, 21]. The loss of the integrity of cell-mediated immune responses allows ubiquitous environmental organisms with limited virulence to become life threatening pathogens [6]. Examples of conditions (as set out by the US Centers for Disease Control and Prevention) include candidiasis of bronchi, trachea, lungs or oesophagus, invasive cervical cancer, coccidioidomycosis, cryptococcosis, cryptosporidiosis, cytomegalovirus disease (other than liver, spleen, or nodes), cytomegalovirus retinitis (with loss of vision), HIV-related encephalopathy, herpes simplex, histoplasmosis, isosporiasis, Kaposi's sarcoma, Burkitt's lymphoma, immunoblastic lymphoma, primary lymphoma of the brain, *Mycobacterium avium* complex, *Mycobacterium tuberculosis*, *Pneumocystis jirovecii* pneumonia, recurrent pneumonia, progressive multifocal leukoencephalopathy,

recurrent salmonella septicaemia, toxoplasmosis of the brain, and wasting syndrome due to HIV [21].

EPIDEMIOLOGY: HIV is a major global problem with approximately 25 million HIV-related deaths and another 40.3 (36 to 45.3) million infected individuals worldwide [17]. AIDS was first described in 1981. The new retrovirus (HIV-1) was found in tissues from AIDS patients in 1983 and the causative relationship between HIV and AIDS was established in 1984 [3, 12]. HIV-2 was discovered in 1986 and is the least pathogenic form of HIV, displaying low rates of transmission and rarely causing AIDS [4]. The majority of people with HIV live in the developing world (approximately 95% of the individuals infected worldwide). Sub-Saharan Africa is by far the worst-affected area in the world [10]. This region has slightly more than 10% of the world's population but is home to more than 60% of the total population living with HIV/AIDS [10].

Globally, infants who acquire the disease from their mothers constitute about 11% of all HIV infections [10]. Ten percent of infections worldwide are associated with injection drug use; 5 to 10% are transmitted by sex between men; and 5 to 10% occur in health care settings [10]. The predominant means of infection is sex between men and women, which accounts for nearly two thirds of new infections, and 85% of existing infections worldwide [10, 17]. About 50% of all new HIV infections worldwide occur in individuals younger than 25 years old [10].

HOST RANGE: Humans [3-6, 8, 10-13, 15-17, 20-23].

INFECTIOUS DOSE: Unknown.

MODES OF TRANSMISSION: HIV is transmitted either by exposure of the virus to oral, rectal, or vaginal mucosa during sexual activity; by intravascular inoculation through transfusion of contaminated blood products; by using contaminated equipment during injection drug use; or from mother to infant during pregnancy, delivery or breastfeeding [6, 16]. There are no obvious differences in disease manifestations in individuals infected by mucosal versus blood-borne routes [6]. Sexual transmission accounts for more than 90% of HIV infections worldwide [6, 16].

INCUBATION PERIOD: Variable. Commonly the time from infection to the development of detectable antibodies is generally 1 to 3 months; however, the time from HIV infection to diagnosis of AIDS had an observed range of less than 1 year to 15 years or longer [11].

COMMUNICABILITY: The highest levels of per-act risk for HIV transmission from person-to-person are: blood transfusion from an infected donor, needle sharing by infected injection-drug users, receptive anal intercourse, and percutaneous needle injuries [6, 11, 12, 20]. Insertive anal intercourse, penile-vaginal exposures, and oral sex represent substantially less per-act risk [6, 11, 20]. HIV can also be passed from mother to child *in utero* (vertical) as well as during childbirth, and from breast milk [6, 11]. HIV has also been documented to have been transmitted by bite injuries [22].The period of communicability begins early after HIV infection and is thought to last throughout the life of the infected individual [11]. Infectiousness is related to viral load.

SECTION III - DISSEMINATION

RESERVOIR: Humans [6, 8, 10-12, 16, 17, 22].

ZOONOSIS: None, although current evidence suggests that HIV-1 and HIV-2 entered into the human population through multiple zoonotic infections from simian immunodeficiency virus-infected non-human primates [17].

VECTORS: No laboratory or epidemiological evidence suggests that biting insects have transmitted HIV infection [11, 16].

SECTION IV – STABILITY AND VIABILITY

DRUG SUSCEPTIBILITY: Antiretroviral agents from 5 drug classes are currently available to treat HIV infection, namely: the nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), proteinase inhibitors (PIs), and fusion inhibitors [10, 15].

SUSCEPTIBILITY TO DISINFECTANTS: HIV is susceptible to fresh 2% glutaraldehyde, 2% Jodopax (detergent and iodine), hypochlorite, iodine, phenolics, and to a lesser extent 70% ethanol, NaOH and isopropanol [7, 9, 18].

PHYSICAL INACTIVATION: HIV is inactivated by ultraviolet (UV) light; however, the level of the inactivation is heavily influenced by the proximity of the UV source to the sample and the concentration of protein in the sample environment. HIV is easily inactivated in a cell free medium; however, in cell associated samples and blood samples complete inactivation requires much longer exposures to the UV source [7]. HIV is also inactivated at pH higher or lower than the optimal level of 7.1 [18]. A temperature of 60°C for 30 minutes will likely inactivate HIV; however, higher temperatures and incubations may be required depending on the initial titre of the virus [18].

SURVIVAL OUTSIDE HOST: HIV can remain viable in blood in syringes at room temperature for 42 days, and in blood and cerebrospinal fluid from autopsies for up to 11 days [1, 2]. Although drying in the environment is known to cause a rapid reduction in HIV concentration, under experimental conditions, Cell-free HIV dried onto a glass coverslip in 10% serum can survive for longer than 7 days, depending on the initial titre [19].

SECTION V - FIRST AID / MEDICAL

SURVEILLANCE: HIV is diagnosed by tests that assess whether an individual's immune system has produced an HIV-specific immune response [16]. Common tests include the indirect binding assay, antibody capture assay, the double antigen sandwich, ELISA, immunofluorescence, Western blotting, line immunoassays, and PCR, as well as viral isolation [16].

FIRST AID/TREATMENT: AIDS must be managed as a chronic disease. Antiretroviral treatment is complex, involving a combination of drugs and resistance will appear rapidly if only a single drug is used [11]. The 5 available classes of antiretroviral drugs, NRTIs, NtRTIs, NNRTIs, PIs and fusion inhibitors, can be combined to provide highly active antiretroviral therapy (HAART). For many (but not all) patients, HAART converts an inexorably fatal disease into a chronic disease with a fairly good prognosis [8, 13].

IMMUNIZATION: None.

PROPHYLAXIS: HIV postexposure prophylaxis regimens are based on the nature of the exposure. The majority of HIV exposures will warrant a two drug regimen, using 2 NRTIs or 1 NRTI and 1 NtRTI. Combinations include: zidovudine (ZDV) and lamivudine (3CT) or emtricitabine (FTC); stavudine (d4T) and 3TC or FTC; and tenofovir (TDF) and 3TC or FTC [15].

The addition of a third or fourth drug should be considered for exposures that pose an increased risk of transmission. The preferred drugs in this case are proteinase inhibitors such as lopinavir/ritonavir (LPV/RTV) [15, 16].

SECTION VI - LABORATORY HAZARD

LABORATORY-ACQUIRED INFECTIONS: Although there have been many reported cases of HIV infection through occupational transmission, the numbers of laboratory acquired infections are low. As of 2001, there have been a total of 57 cases of documented occupationally acquired HIV among U.S. health care workers [24].

SOURCES/SPECIMENS: Blood, semen, vaginal secretions, cerebrospinal fluid, synovial fluid, peritoneal fluid, pleural fluid, pericardial fluid, amniotic fluid, other specimens containing visible blood, breast milk,

unscreened or inadequately treated blood products, and infected human tissues [11, 15, 16].

Faeces, nasal secretions, sputum, sweat, vomitus, saliva, tears, and urine, are not considered potentially infectious unless they are visibly bloody [11, 15].

PRIMARY HAZARDS: Needlestick, contaminated sharp objects, and/or direct contact of non-intact skin or mucous membranes with HIV-infected specimens/tissues [15, 16].

SPECIAL HAZARDS: Extreme care must be taken to avoid spilling and/or splashing infected materials. HIV should be presumed to be in/on all equipment and devices coming in direct contact with infected materials [25].

SECTION VII - EXPOSURE CONTROLS / PERSONAL PROTECTION

RISK GROUP CLASSIFICATION: Risk Group 3 [26].

CONTAINMENT REQUIREMENTS: Containment Level 2 facilities and equipment for work involving clinical specimens and non-culture procedures. Containment Level 3 facilities, equipment, and operational practices for all work culturing HIV and for activities involving non-human primates and any animals experimentally infected or inoculated with HIV [25].

PROTECTIVE CLOTHING: Solid-front gowns with tight-fitting wrists, gloves, and respiratory protection should be worn over laboratory clothing when infectious materials are directly handled [25].

OTHER PRECAUTIONS: All activities with infectious material should be conducted in a biological safety cabinet (BSC) or other appropriate primary containment device in combination with personal protective equipment. Centrifugation of infected materials must be carried out in closed containers placed in sealed safety cups, or in rotors that are unloaded in a biological safety cabinet. The use of needles, syringes, and other sharp objects should be strictly limited. Open wounds, cuts, scratches, and grazes should be covered with waterproof dressings. Additional precautions should be considered with work involving animals or large scale activities [25].

SECTION VIII - HANDLING AND STORAGE

SPILLS: Allow aerosols to settle and, while wearing protective clothing, gently cover the spill with paper towels and apply 1% sodium hypochlorite starting at the perimeter, working inwards towards the centre. Allow sufficient contact time before clean up [25] .

DISPOSAL: Decontaminate all materials for disposal by steam sterilisation, chemical disinfection, and/or incineration [25].

STORAGE: Infectious material should be stored in sealed, leak-proof containers that are appropriately labelled [25].

SECTION IX - REGULATORY AND OTHER INFORMATION

REGULATORY INFORMATION: The import, transport, and use of pathogens in Canada is regulated under many regulatory bodies, including the Public Health Agency of Canada, Health Canada, Canadian Food Inspection Agency, Environment Canada, and Transport Canada. Users are responsible for ensuring they are compliant with all relevant acts, regulations, guidelines, and standards.

UPDATED: September 2011.

PREPARED BY: Pathogen Regulation Directorate, Public Health Agency of Canada.

Although the information, opinions and recommendations contained in this Material Safety Data Sheet are compiled from sources believed to be reliable, we accept no responsibility for the accuracy, sufficiency, or

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Date Modified: 2011-09-08

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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* deposited as human

Morphology: epithelial

Source: **Organ:** kidney

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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 Rockefeller University, Office of Technology Transfer, 1230 York Avenue, New York, NY 10065 Attn: Kathleen A. Denis, Associate Vice President Technology Transfer.

Applications: 293T cells were cloned and the clones tested with the pBND and pZAP

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vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.

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

Comments: The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability. 293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line. ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line. ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.

Propagation:

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

Subculturing:

Protocol:

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

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 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

derivative:ATCC CRL-11269

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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

ALL RECIPIENTS OF THIS MATERIAL MUST COMPLY WITH ALL APPLICABLE BIOLOGICAL, CHEMICAL, AND/OR RADIOCHEMICAL SAFETY STANDARDS INCLUDING SPECIAL PRACTICES, EQUIPMENT, FACILITIES, AND REGULATIONS. NOT FOR USE IN HUMANS.

Sterility:	Negative for bacteria, mycoplasma, and fungi.
Recommended Storage:	Liquid nitrogen.
Contributor:	Dr. Peter L. Nara.
Description:	Human T4-lymphoblastoid cell line initially derived by G.E Foley et al. and biologically cloned by P.L. Nara et al.
References:	Foley GE, Lazarus H, Farber S, Uzman BG, Boone BA, McCarthy RE. Continuous culture of human lymphoblasts from peripheral blood of a child with acute leukemia. *Cancer* **18**:522-529, 1965. Nara PL, Hatch WC, Dunlop NM, Robey WG, Fischinger PJ. Simple, rapid quantitative, syncytium-forming microassay for the detection of human immunodeficiency virus neutralizing antibody. *AIDS Res Hum Retroviruses* **3**:283-302, 1987. Nara PL, Fischinger PJ. Quantitative infectivity assay for HIV-1 and -2. *Nature* **332**:469-470, 1988.
NOTE:	Acknowledgment for publications should read "The following reagent was obtained through the NIH AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH: CEM-SS (Cat# 776) from Dr. Peter L. Nara." Please include the references cited above in any publications.
Last Updated	August 12, 2010

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Cell Biology

ATCC® Number: HTB-176™ Order this Item

Price: $431.00 (for-profit list price)
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Designations:	H9 [derivative of HuT 78]
Depositors:	RC Gallo, M Popovic
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens*
Morphology:	lymphoblast
	PHOTO
Source:	**Disease:** lymphoma **Cell Type:** cutaneous T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2)
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)
Tumorigenic:	Yes
Antigen Expression:	CD4; HLA A1, B62, C3, DR4, DQ3
DNA Profile (STR):	Amelogenin: X,Y

Related Links

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- Technical Support
- Related Cell Culture Products

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

Cytogenetic Analysis: This is a near triploid cell line (modal number = 69; range = 58 to 74). The frequency of higher ploidies is 2.5%. The line has an extremely complex karyotype with nearly 60% of the chromosomes in each cell being structurally altered marker chromosomes., Among the markers are t(3p4q), t(5q6q), t(5p6p), i(18q), i(18p); t(4q7p), and del(7)(q32). The first four of these are usually paired. Normal N4, N5, N6, N7, N10, N13, N18, N19, N20 an X are absent.

Isoenzymes: AK-1, 0
ES-D, 1
G6PD, B
GLO-I, 1
Me-2, 0
PGM1, 1
PGM3, 0

Age: 53 years

Gender: male

Ethnicity: Caucasian

Comments: The H9 cell line is a clonal derivative of the Hut 78 cell line (see ATCC TIB-161).
The H9 clone was selected for permissiveness for HIV-1 replication, and has been used to isolate and propagate HIV-1 from the blood of patients with acquired immunodeficiency syndrome (AIDS) and pre-AIDS conditions.

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%.
Temperature: 37.0°C

Subculturing:

Medium Renewal: Every 2 to 4 days
Cultures can be maintained by addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension in fresh medium at 5 x 10 exp5 viable cells/ml.
Maintain cultures at cell concentrations between 5 X 10 exp5 and 2 X 10 exp6 viable cells/ml.
Do not allow cell concentration to exceed 3 X 10 exp6 cells/ml.

Preservation: Culture medium, 95%; DMSO, 5%

Related Products: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001
recommended serum:ATCC 30-2020
parental cell line:ATCC TIB-161

References:
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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*

Morphology: epithelial

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

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
screening for Escherichia coli strains with invasive potential

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

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Product Information Sheet

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CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10
D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

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

Isoenzymes:
G6PD, A

Age:
31 years adult

Gender:
female

Ethnicity:
Black

HeLa Markers:
Y

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

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37°C

Subculturing:

Protocol:

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

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

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

Related Products:
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003

also available as Certified Reference Material, ATCC CRM-CCL-2
derivative:ATCC CCL-2.1
derivative:ATCC CCL-2.2
derivative:ATCC CCL-2.3

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Designations:	Jurkat, Clone E6-1
Depositors:	A Weiss
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens*
Morphology:	lymphoblast
	PHOTO
Source:	**Disease:** acute T cell leukemia **Cell Type:** T lymphocyte;
Cellular Products:	interleukin-2 (interleukin 2, IL-2)
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:	T cell antigen receptor, expressed
Antigen Expression:	CD3; Homo sapiens, expressed
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12

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Product Information Sheet

D13S317: 8,12
D16S539: 11
D5S818: 9
D7S820: 8,12
THO1: 6,9.3
TPOX: 8,10
vWA: 18

Cytogenetic Analysis: This is a pseudodiploid human cell line. The modal chromosome number is 46, occurring in 74% with polyploidy at 5.3%. The karyotype is 46,XY,-2,-18,del(2) (p21p23),del(18) (p11.2). Most cells had normal X and Y chromosomes.

Gender: male

Comments: This is a clone of the Jurkat-FHCRC cell line, a derivative of the Jurkat cell line.
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.
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.
The line was cloned from cells obtained from Dr. Kendall Smith and are mycoplasma free.

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

References:

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A better way to share plasmids

PLASMID MAPS

Browse > Didier Trono > Trono Lab Packaging and Envelo... > pCMV delta R8.2

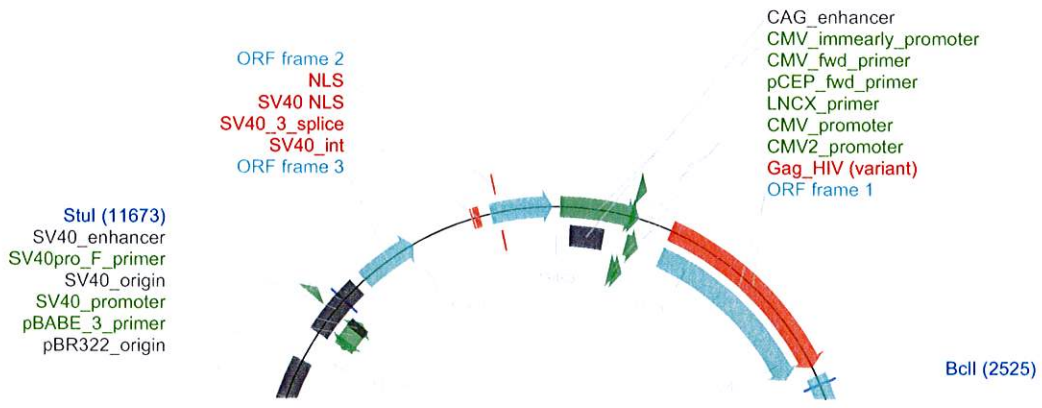
Plasmid 12263: pCMV delta R8.2

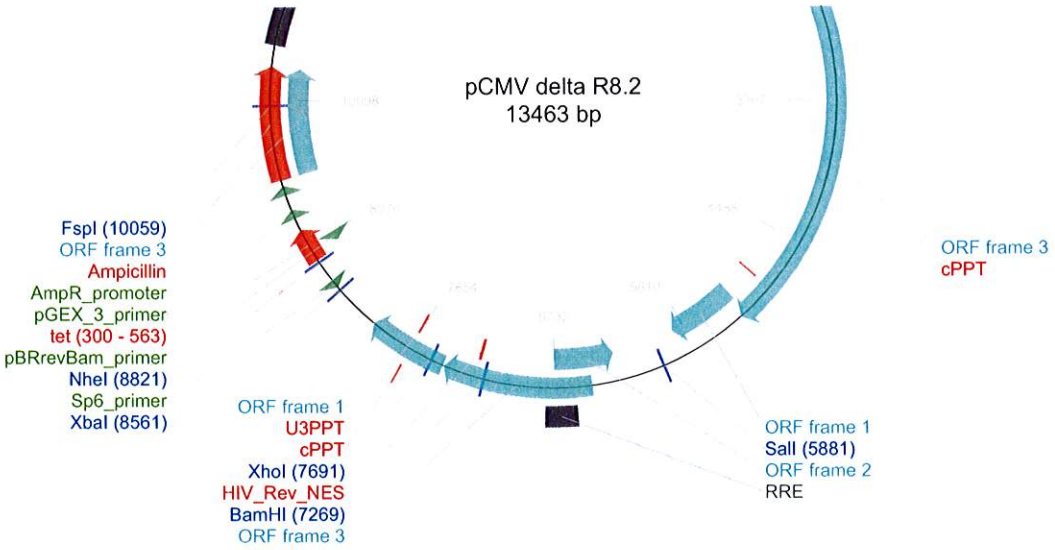
Gene/insert name:	HIV-1 GAG/POL, Tat and Rev
Vector backbone:	pCMVR8.2 (Search Vector Database)
Vector type:	Mammalian Expression, Lentiviral
Vector type:	Packaging
Backbone size w/o insert (bp):	8128
5' sequencing primer:	CMV forward List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Sequence:	View sequences (2)
Supplemental document:	Digest Plasmid 12263 (application/pdf)
Principal Investigator:	Didier Trono
Terms and Licenses:	MTA

Comments: Packaging plasmid.

Please note that the full sequence for this plasmid is approximated and not fully verified. Please visit the Trono lab http://tronolab.epfl.ch for cloning strategies, protocols, publications, and more. See LentiWeb http://www.lentiweb.com for discussion on cloning strategies and protocols.

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





Feature Name	Start	End
CMV_immearly_promoter	27	603
CAG_enhancer	106	393
CMV_fwd_primer	560	580
CMV_promoter	561	630
CMV2_promoter	573	692
pCEP_fwd_primer	604	623
LNCX_primer	606	630
Gag_HIV (variant)	880	2388
cPPT	4881	4896
RRE	6563	6796
HIV_Rev_NES	7319	7348
cPPT	7863	7878
U3PPT	7863	7884
Sp6_primer	8600	8583
tet (300 - 563)	8839	9102
pBRrevBam_primer	8910	8891
pGEX_3_primer	9244	9266
AmpR_promoter	9425	9453
Ampicillin	9495	10355
pBR322_origin	10510	11129
pBABE_3_primer	11375	11355
SV40_enhancer	11814	11361
SV40_promoter	11373	11641
SV40_origin	11540	11617
SV40pro_F_primer	11602	11621
SV40_int	12823	12838
SV40_3_splice	12844	12891
SV40 NLS	13019	13039
NLS	13025	13039

ORF	Start	End
ORF frame 1	880	2388

ORF frame 3	2454	5192
ORF frame 1	5137	5715
ORF frame 2	6736	6233
ORF frame 3	6441	7589
ORF frame 1	7591	8211
ORF frame 3	9495	10355
ORF frame 3	11889	12347
ORF frame 2	12968	13441

Enzyme Name	**Cut**
BclI	2525
SalI	5881
BamHI	7269
XhoI	7691
XbaI	8561
NheI	8821
FspI	10059
StuI	11673

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 12263" in your Materials and Methods section.

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A better way to share plasmids

Browse > Bob Weinberg > Stewart et al > pCMV-VSV-G

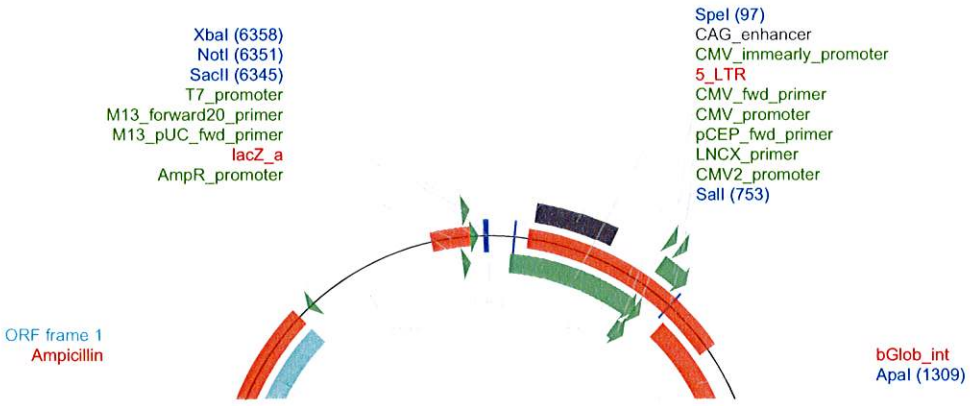
Plasmid 8454: pCMV-VSV-G

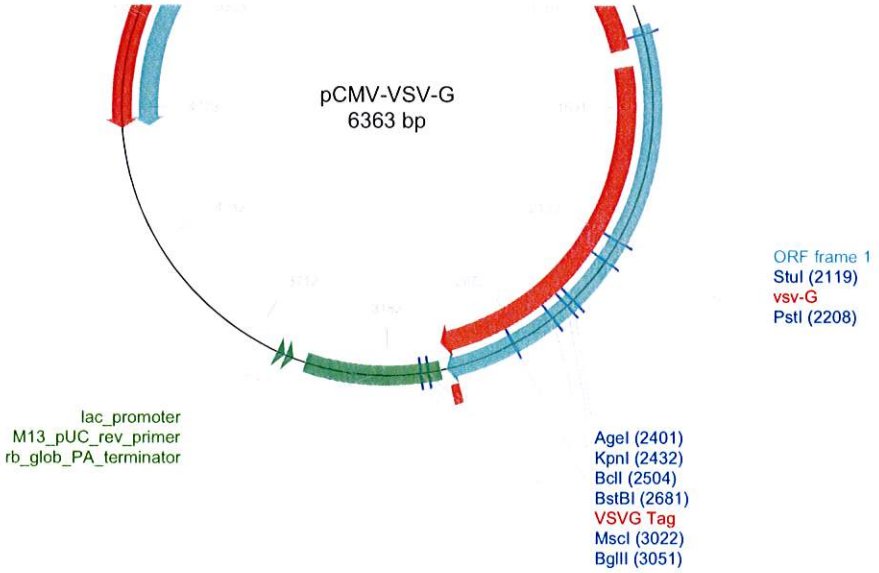
Gene/insert name:	None
Vector backbone:	na (Search Vector Database)
Vector type:	Mammalian Expression
Backbone size (bp):	6363
5' sequencing primer:	T7 List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Sequence:	View sequences (4)
Supplemental document:	Weinberg-viral (application/pdf)
Supplemental document:	Notes from Addgene (2)
Principal Investigator:	Bob Weinberg
Terms and Licenses:	MTA

Comments: VSVG envelope protein, for use with lentiviral and MuLV vectors. The 293T cell line can be obtained from the Weinberg lab or GenHunter http://genhunter.com/products/aptag-3/index.html

Please note that depositor's sequence is not 100% complete. The flanking sequence of VSV-G actually contains XhoI (at both the 5' and 3'end). You can see sequence for this area in the Reviews link to the right..

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





Feature Name	Start	End
CMV_immearly_promoter	84	660
5_LTR	148	974
CAG_enhancer	163	450
CMV_fwd_primer	617	637
CMV_promoter	618	687
CMV2_promoter	630	749
pCEP_fwd_primer	661	680
LNCX_primer	663	687
bGlob_int	778	1350
vsv-G	1420	2955
VSVG Tag	2920	2952
rb_glob_PA_terminator	2974	3500
M13_pUC_rev_primer	3567	3545
lac_promoter	3610	3581
Ampicillin	5553	4693
AmpR_promoter	5623	5595
lacZ_a	6295	6140
M13_pUC_fwd_primer	6266	6288
M13_forward20_primer	6281	6297
T7_promoter	6307	6325

ORF	Start	End
ORF frame 1	1276	2955
ORF frame 1	5553	4693

Enzyme Name	Cut
SpeI	97
SalI	753
ApaI	1309
StuI	2119
PstI	2208
AgeI	2401

KpnI	2432
BclI	2504
BstBI	2681
MscI	3022
BglII	3051
SacII	6345
NotI	6351
XbaI	6358

Article: Lentivirus-delivered stable gene silencing by RNAi in primary cells. Stewart et al (RNA 2003 Apr;9(4):493-501. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 8454" in your Materials and Methods section.

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A better way to share plasmids

Browse > Bob Weinberg > Stewart et al > pLKO.1 puro

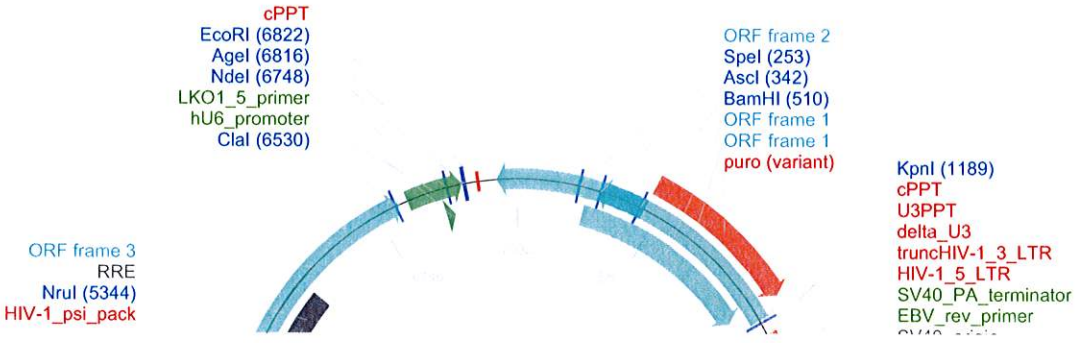
Plasmid 8453: pLKO.1 puro

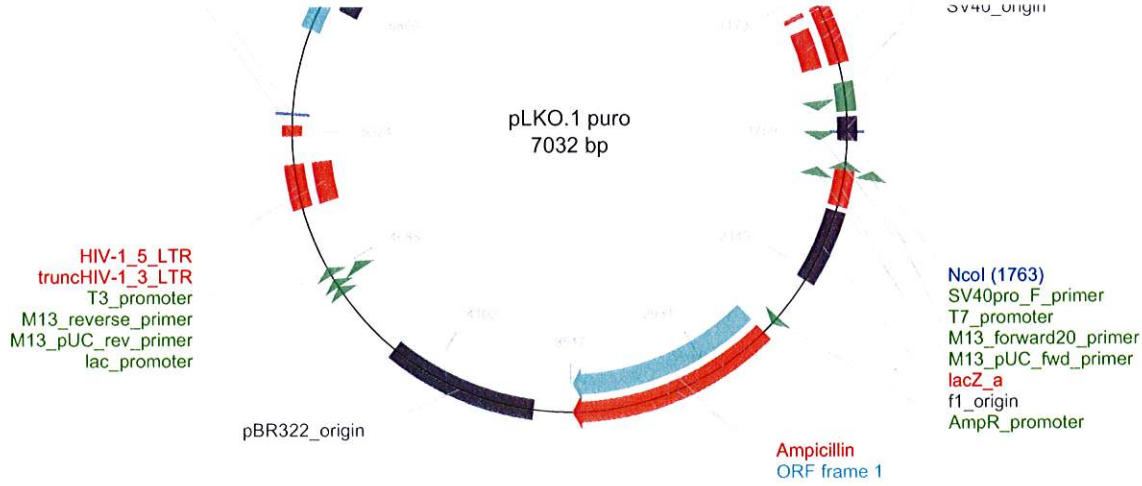
Gene/insert name:	None
Alt name:	LKO.1
Vector backbone:	pLKO.1 (Search Vector Database)
Vector type:	Mammalian Expression, Lentiviral, RNAi
Backbone size (bp):	7032
Cloning site 5':	AgeI
Site destroyed during cloning:	No
Cloning site 3':	EcoRI
Site destroyed during cloning:	No
5' sequencing primer:	LKO.1 5' List of Sequencing Primers
Bacterial resistance(s)	Ampicillin
Growth strain(s)	DH5alpha
Growth temperature (°C):	37
High or low copy:	High Copy
Selectable markers:	Puromycin
Sequence:	View sequences (2)
Supplemental document:	Addgene's pLKO.1 protocol
Supplemental document:	Notes from Addgene (1)
Principal Investigator:	Bob Weinberg
Terms and Licenses:	MTA

Comments: Empty lentiviral vector for siRNA expression; replaces Lentihair; see author's map. The 293T cell line can be obtained from the Weinberg lab or GenHunter http://genhunter.com/products/aptag-3/index.html

For packaging, please use pCMV-dR8.2 dvpr (Addgene plasmid #8455) and pCMV-VSVG (Addgene plasmid #8454). For the official vector of The RNAi Consortium and a plasmid map, please see plasmid #10878.

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





Feature Name	Start	End
puro (variant)	529	1128
U3PPT	1239	1260
cPPT	1239	1254
delta_U3	1256	1308
truncHIV-1_3_LTR	1309	1489
HIV-1_5_LTR	1309	1489
SV40_PA_terminator	1564	1683
EBV_rev_primer	1652	1671
SV40_origin	1701	1799
SV40pro_F_primer	1784	1803
T7_promoter	1896	1878
M13_forward20_primer	1922	1906
M13_pUC_fwd_primer	1937	1915
lacZ_a	1915	2063
f1_origin	2081	2387
AmpR_promoter	2580	2608
Ampicillin	2650	3510
pBR322_origin	3665	4284
lac_promoter	4593	4622
M13_pUC_rev_primer	4636	4658
M13_reverse_primer	4657	4675
T3_promoter	4692	4711
truncHIV-1_3_LTR	4965	5145
HIV-1_5_LTR	4965	5145
HIV-1_psi_pack	5256	5300
RRE	5810	6043
hU6_promoter	6579	6811
LKO1_5_primer	6741	6760
cPPT	6867	6882

ORF	Start	End
ORF frame 1	289	1128

ORF frame 1	1179	334
ORF frame 1	2650	3510
ORF frame 3	5688	6545
ORF frame 2	530	6948

Enzyme Name	**Cut**
SpeI	253
AscI	342
BamHI	510
KpnI	1189
NcoI	1763
NruI	5344
ClaI	6530
NdeI	6748
AgeI	6816
EcoRI	6822

Article: Lentivirus-delivered stable gene silencing by RNAi in primary cells. Stewart et al (RNA 2003 Apr;9(4):493-501. PubMed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication. Also, please include the text "Addgene plasmid 8453" in your Materials and Methods section.

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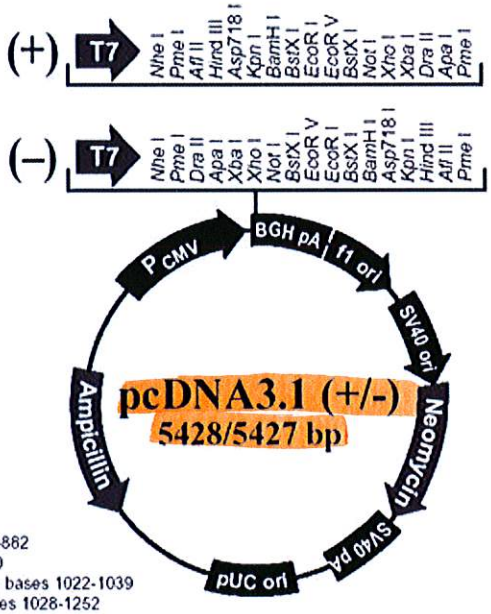

Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

pcDNA3.1(-) Restriction map

pcDNA3.1(+) Restriction map

Resources

Plasmid Files

pEYFP-N1

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

pEYFP-N1.dna (Sequence and Map File | 34 KB)
Sequence Author: Clontech

SnapGene Viewer 1.2 for Mac OS X (43 MB)

SnapGene Viewer 1.2 for Windows (13 MB)

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Fluorescent Protein Genes & Plasmids
pEYFP-N1
Yeast Plasmids
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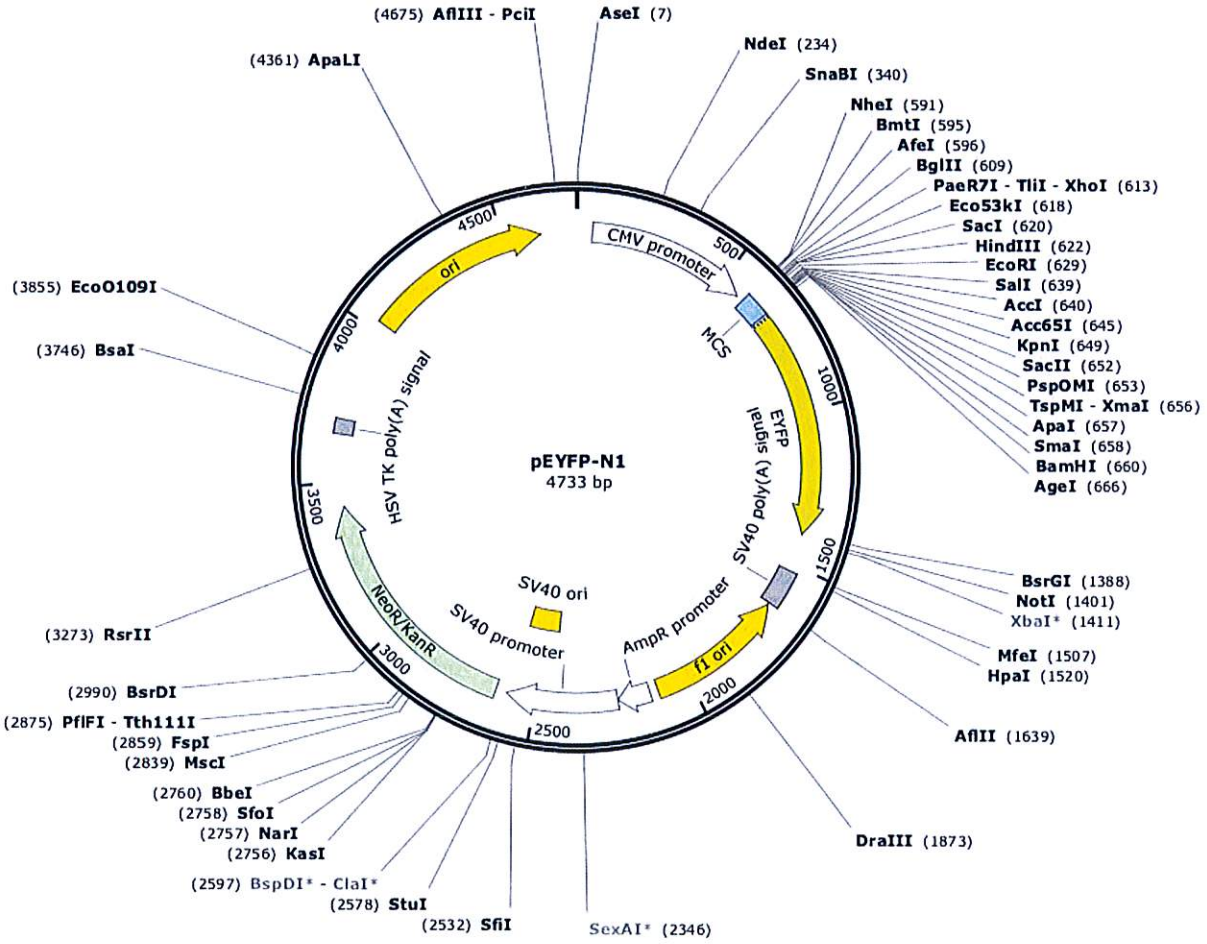
Qiagen Vectors



Individual Sequences & Maps

AcGFP1	hKO	pAcGFP1-C1	pECFP-1	pmCherry-N1	ptdTomato
Azami-Green	hmAzami-Green	pAcGFP1-C2	pECFP-C1	pMiCy1-S1	ptdTomato-C1
Azurite BFP	hMGFP	pAcGFP1-C3	pECFP-N1	pmKate2-C	pTimer
BFP	hmKeima-Red	pAcGFP1-N1	pEGFP	pmKate2-N	pTimer-1
CFP	hmKikGR1	pAcGFP1-N2	pEGFP-1	pmKeima-Red-S1	pTurboFP602-B
Citrine	hmKO	pAcGFP1-N3	pEGFP-C1	pmKikGR1-S1	pTurboFP602-C
Cycle 3 GFP	hmKO2	pAG-S1	pEGFP-C2	pmKO1-S1	pTurboFP602-N
CyPet	hmMiCy1	PAmCherry	pEGFP-C3	pmKO2-S1	pTurboFP602-PRL
CyPet (humanized)	hmUkG1	pAmCyan	pEGFP-N1	pmMiCy1-S1	pTurboGFP-B
d1EGFP	Kaede	pAmCyan1-C1	pEGFP-N2	pmOrange	pTurboGFP-C
d2ECFP	KikGR1	pAmCyan1-N1	pEGFP-N3	pmOrange2	pTurboGFP-N
d2EGFP	KillerRed	pAsRed2	pEYFP	pmOrange2-C1	pTurboGFP-PRL
d2EYFP	Kusabira-Orange	pAsRed2-C1	pEYFP-1	pmOrange2-N1	pTurboRFP-B
d4EGFP	mApple	pAsRed2-N1	pEYFP-C1	pmPlum	pTurboRFP-C
Dendra2	mAzami-Green	pd1EGFP-N1	pEYFP-N1	pmRaspberry	pTurboRFP-N
dKeima-Red	mCerulean	pd2ECFP-N1	pGFP	pmStrawberry	pTurboRFP-PRL
dKeima570	mCherry	pd2EGFP-N1	pGFPuv	pmUkG1-S1	pTurboYFP-B
Dronpa-Green1	mECFP	pd2EYFP-N1	pGLO	pNirFP-C	pTurboYFP-C
Dronpa-Green3	mEGFP	pd4EGFP-N1	pHcRed1	pNirFP-N	pTurboYFP-N
DsRed-Express	mEos2	pDendra2	pHcRed1-1	pPA-TagRFP-C	pTurboYFP-PRL
DsRed-Express2	mEYFP	pDendra2-C	pHcRed1-C1	pPA-TagRFP-N	pZsGreen
DsRed-Max	mHoneydew	pDendra2-N	pHcRed1-N1_1	pPAmCherry-C1	pZsGreen1-1
DsRed-Monomer	MiCy	pDG1-S1	phdKeima-Red-S1	pPAmCherry-N1	pZsGreen1-C1
DsRed.T3	mKate2	pDG3-S1	phdKeima570-S1	pPhi-Yellow-B	pZsGreen1-N1
DsRed1	mKeima-Red	pdKeima-Red-S1	phKikGR1-S1	pPhi-Yellow-C	pZsYellow
DsRed2	mKikGR1	pdKeima570-S1	phKO1-S1	pPhi-Yellow-N	pZsYellow1-C1
dTomato	mKO	pDsRed-Express	phmAG1-S1	pPhi-Yellow-PRL	pZsYellow1-N1
E2-Crimson	mKO2	pDsRed-Express-1	phMGFP	pPS-CFP2-C	superfolder GFP
E2-Orange	mMiCy1	pDsRed-Express-C1	phmKeima-Red-S1	pPS-CFP2-N	TagBFP
E2-Red_Green	mOrange	pDsRed-Express-N1	phmKO1-S1	pRSET-BFP	TagCFP
EBFP	mOrange2	pDsRed-Express2	phmUkG1-S1	pRSET-CFP	TagGFP2
EBFP2	mPlum	pDsRed-Express2-1	pKaede-S1	pRSET-EmGFP	TagRFP
ECFP	mRaspberry	pDsRed-Express2-C1	pKikGR1-S1	PS-CFP2	TagRFP-T
EGFP	mRFP1	pDsRed-Express2-N1	pKillerRed-B	pTagBFP-C	TagYFP
Emerald GFP	mRuby	pDsRed-Monomer-C1	pKillerRed-C	pTagBFP-N	tdTomato
EosFP	mTangerine	pDsRed-Monomer-N1	pKillerRed-N	pTagCFP-C	TurboFP602
EYFP	mTFP1	pDsRed2	pKindling-Red-B	pTagCFP-N	TurboGFP
Fluorescent Timer	mTurquoise	pDsRed2-1	pKindling-Red-N	pTagGFP2-C	TurboRFP
GFP	mTurquoise2	pDsRed2-C1	pKO1-S1	pTagGFP2-N	TurboYFP
GFPuv	mUkG1	pDsRed2-N1	pmAG1-S1	pTagRFP-C	YFP
HcRed1	mVenus	pE2-Crimson	pmBanana	pTagRFP-N	YPet
hdKeima-Red	mWasabi	pE2-Crimson-C1	pmCherry	pTagYFP-C	YPet (humanized)
hdKeima570	pAcGFP1	pE2-Crimson-N1	pmCherry-1	pTagYFP-N	ZsGreen1
hKikGR1	pAcGFP1-1	pECFP	pmCherry-C1	ptd-Tomato-N1	ZsYellow1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pGEX-1λT (27-4805-01)

Thrombin

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

BamH I EcoR I Stop codons

pGEX-2T (27-4801-01)

Thrombin

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

BamH I Sma I EcoR I Stop codons

pGEX-2TK (27-4587-01)

Thrombin Kinase

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

BamH I Sma I EcoR I Stop codons

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

Thrombin

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

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

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

Thrombin

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

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

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

Thrombin

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

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

pGEX-3X (27-4803-01)

Factor Xa

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

BamH I Sma I EcoR I Stop codons

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

Factor Xa

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

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

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

Factor Xa

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

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

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

Factor Xa

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

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

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

PreScission™ Protease

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

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

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

PreScission™ Protease

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

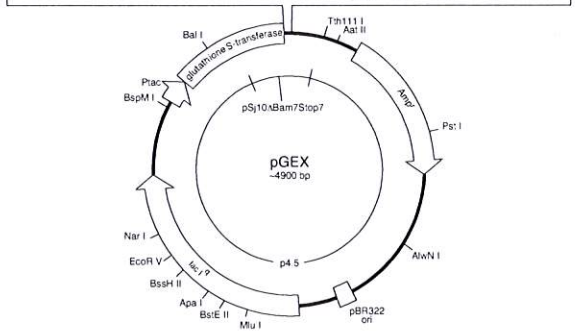
BamH I EcoR I Sma I Sal I Xho I Not I

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

PreScission™ Protease

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

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



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

pET-41a-c(+) Vector

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

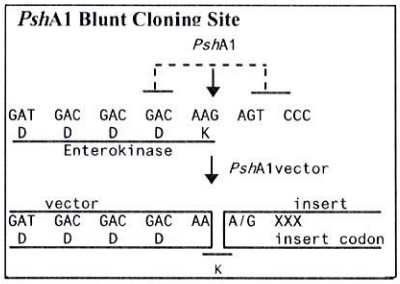
pET-41a(+) sequence landmarks

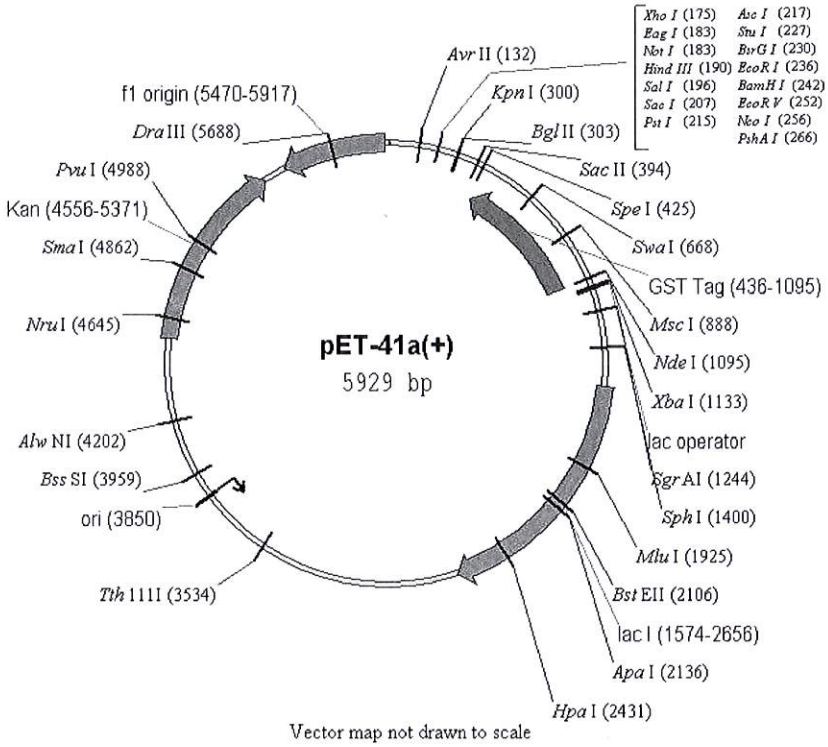
T7 promoter	1167-1183
T7 transcription start	1166
GST•Tag coding sequence	436-1095
His•Tag coding sequence	397-414
S•Tag coding sequence	310-354
Multiple cloning sites (*Psh*A 1 - *Xho* I)	175-266
His•Tag coding sequence	150-173
T7 terminator	26-72
lacI coding sequence	1574-2656
pBR322 origin	3850
kan coding sequence	4556-5371
F1 origin	5470-5917

Notes

All corresponding maps and tables are generated using VectorNTI® software. Restriction sites are numbered in accordance with the conventions of the respective software.

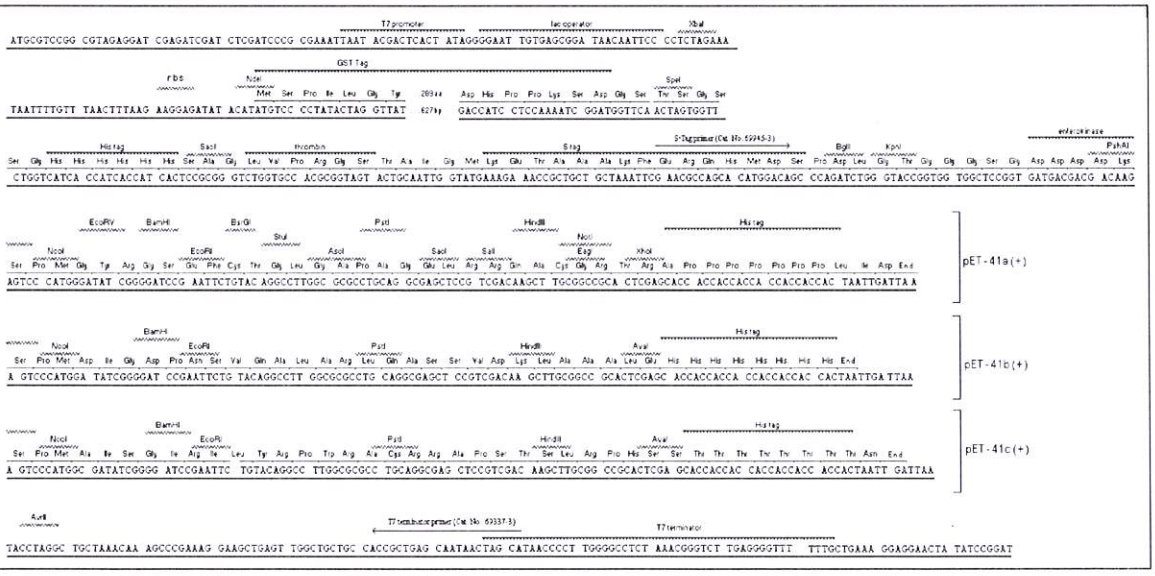
The pET-41 series is designed for cloning and high-level expression of peptide sequences fused with the 220 aa GST•Tag™ protein. Unique sites are shown on the circle map. Note that the sequence is numbered by the pBR322 convention, so the T7 expression region is reversed on the circle map. The cloning/expression 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.





Vector map not drawn to scale

pET-41a-c(+) cloning/expression regions



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Enzyme	#	Locations				
AccI	2	197	3559			
AciI	73					
AflIII	3	853	1925	3786		
AluI	25					
AlwI	12					
AlwNI	1	4202				
ApaI	1	2136				
ApaLI	3	1905	3603	4100		
ApoI	7	236	332	2200	4601	
		4785	5490	5501		
AscI	1	217				
AvaI	2	175	4860			
AvaII	4	598	2477	2795	3074	
AvrII	1	132				
BamHI	1	242				
BanI	9	296	351	1247	1268	1382
		1845	2564	2694	5725	
BanII	6	207	1309	1323	2136	4643
		5763				
BbsI	3	2071	2410	*2907		
BbvI	24					
BcgI	4	*177	1067	2251	*3366	
BclI	2	657	1939			
BfaI	10	71	133	426	1081	1134
		2768	2803	4281	4588	5839
BglII	1	303				
BpmI	3	*1763	2252	3316		
Bpu10I	2	2895	*5005			
BsaAI	2	3541	5688			
BsaBI	3	1198	1208	2986		
BsaHI	5	1248	1269	1383	1882	2565
BsaJI	12					
BsaWI	9	3	281	293	2244	2747
		2978	3992	4139	5123	
BsgI	4	*811	*1776	*1976	*2949	
BsiEI	5	186	2710	3702	4126	4988
BsiHKAI	7	176	207	1425	1909	2783
		3607	4104			
BslI	28					
BsmBI	3	2540	*3430	5004		
BsmFI	5	274	1104	1386	*3060	*5903
Bsp1286I	12					
BspEI	2	3	2978			
BsrI	20					
BsrBI	5	804	1154	*3719	*5387	*5832
BsrDI	3	*1972	2338	*3608		
BsrFI	6	293	1235	1244	1611	4942
		5789				
BsrGI	1	230				
BssHII	2	217	2336			
BssSI	1	*3959				
Bst1107I	1	3559				
BstEII	1	2106				
BstXI	3	1727	1856	1979		

Enzyme	#	Locations				
BstYI	8	242	303	1489	2701	2981
		4427	4436	5237		
Cac8I	43					
ClaI	2	1202	4679			
CviJI	89					
DdeI	11					
DpnI	23					
DraI	2	559	668			
DraIII	1	5688				
DrdI	3	3482	3894	5643		
EaeI	5	183	886	1233	1365	2599
EagI	1	183				
EarI	4	1013	*1543	3870	4900	
EcoNI	3	1064	1060	4900		
EcoO109I	4	54	1060	1358	2795	
EcoRI	1	236				
EcoRV	1	262				
FauI	17					
Fnu4HI	40					
FokI	13					
HaeII	13					
HaeIII	24					
HgaI	12					
HhaI	46					
HincII	2	198	2431			
HindIII	1	190				
HinfI	18					
HpaI	1	2431				
HphI	22					
KpnI	1	300				
MaeIII	16					
MboII	15					
MluI	1	1925				
MnlI	24					
MscI	1	888				
MseI	31					
MslI	8	1001	1977	2265	2295	2776
		3362	3613			
MspA1I	10	85	345	393	1955	2525
		2618	3380	3499	4128	4373
MwoI	36					
NarI	4	1248	1269	1383	2565	
NciI	12					
NcoI	1	256				
NdeI	1	1095				
NlaIII	29					
NlaIV	22					
NotI	1	183				
NruI	1	4645				
NsiI	2	4838	5104			
NspI	5	857	1400	3134	3426	3790
PflMI	3	322	1507	5251		
PleI	10					
PshAI	1	266				

Enzyme	#	Locations				
PstI	1	215				
PvuI	1	4988				
PvuII	3	2525	2618	3380		
RsaI	7	232	298	370	522	2072
		3595	4823			
SacI	1	207				
SacII	1	394				
SalI	1	196				
SapI	2	1013	3670			
Sau3AI	23					
Sau96I	13					
ScaI	2	370	522			
ScrFI	22					
SfaNI	24					
SfcI	5	211	1167	4051	4242	5907
SgrAI	1	1244				
SmaI	1	4862				
SpeI	1	425				
SphI	1	1400				
SspI	2	4913	5480			
StuI	1	227				
StyI	4	58	132	222	256	
SwaI	1	668				
TaiI	15					
TaqI	19					
TfiI	8	2604	2839	3343	3761	4899
		4955	5127	5218		
TseI	24					
Tsp45I	6	2106	3228	3441	3536	5136
		5861				
Tsp509I	29					
TspRI	11					
Tth111I	1	3534				
XbaI	1	1133				
XcmI	3	1781	2297	2315		
XhoI	1	175				
XmnI	3	702	3347	5377		

Enzymes that do not cut pET-41a(+):

AatII	AflII	AhdI	BglI	BsaI
BseRI	BspMI	Bsu36I	FseI	FspI
NheI	PacI	PmeI	PmlI	RsrII
SanDI	SexAI	SfiI	SnaBI	SrfI
SunI	UbaEI			

*On complementary strand.

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Experimentation under BSL2 conditions with enhanced safety precautions:

Pseudotyped Human Immunodeficiency Virus-1 (HIV-1):

b. Pseudotype Human Immunodefficiency Virus-1 (HIV-1)

Replication inocompetent HIV-1 vectors will be used to introduce genes that are not known to be growth-altering into human cells for stable expression resulting into gene intergration in the cells. Pseudotyped virus is generated by transfecting three plasmids in the human 293T cell line. The three plasmids are as follow: 1. DeltaR8.2: the packaging plasmid containing genes to produce the structure of the virus. This plasmid lacks essential elements required for replication including long-terminal-repeat (LTR) regions , the genome packaging signal, the envelopple (env) gene and the vpu gene. 2. VSVg , codes for an essential enveloppe protein from (Vesicular Stomatitis Virus) allowing cell entry. This plasmid is provided in trans and will not be packaged in the viruses's new genome. The role of this plasmid is to provide the enveloppe protein essential for viral entry into the target cell. 3. Ppt contains two LTRs and a multiple cloning site in order to insert the gene of choice. This plasmid serves to deliver the gene of choice and is under the control of a CMV promoter. This is the gene that will be integrated into the target cell genome. The resulting virus will be replication incompetent and work will be performed with SDRI 238 under Biosafety 2+3 conditions and enhance safety precautions. All equipments/supplies in contact with the virus will be decontaminated with 10% bleach and subsequently washed with a detergent based soap diluted in water and will be autoclaved.

Since this virus is replication-incompetent, I will perform this procedure in the enclosed tissue culture room (SDRI 238) within my lab (SDRI 237) under Biosafety Level 2+3 conditions with enhanced safety precautions.

The practices that will be carried out are as follows:

Routine procedures for BLS2 with enhanced precautions:

-All personnel will be trained by the principal investigator (Jimmy Dikeakos) prior to having key access to the room and being permitted to perform, assist or visualize work in the room (SDRI 238). The principal investigator will supervise all work a minimum of three times and allow new trainees sole access to the room until he deems them competent to do so.

- During virus work, a clearly labeled sign will be placed on the door of SDRI 238 alerting co- workers that virus work is in progress and that entering personnel should adhere to the safety guidelines outlined below.

- A 10 liter carboy of 70% ethanol and 6 cans of Lysol Industrial Strength disinfectant will be available in the room at all times for disinfecting purposes.

-All vacuum and CO2 hose lines will have approved filters attached.

-Waste traps will contain proper disinfectant (10% bleach) and be set up to include a secondary trap in line with the first trap to collect any residue that travels along the vacuum hose line, thus ensuring no liquid enters the main vacuum line.

-Weekly cleaning and disinfecting of the tissue culture room will be done. This will involve activities such as disinfecting all surfaces, mopping the floor with 1% bleach solution, and refilling all disinfectant.

-Samples that are to be centrifuged will be carried out in centrifuges fitted with biological safety lids. Centrifuge speeds will not exceed 3,000rpm.

-All handling of virus will be performed in the safety hood.

-Virus stocks requiring storage will be stored in locked fridges or freezers and transported to those fridges or freezers in a sealed and properly labeled biological transport container.

-Material coming in contact with virus will be disinfected immediately after use .

Entering the room:

-The door to SDRI 238 will remain closed at all times.

-All personnel will be required to double glove prior to entering even if an experiment is not being performed.

-Personnel will wear self-dedicated tissue culture lab coats that tie from behind.

-Sufficient 10% bleach will be made prior to any experiment for disinfecting spills or anything coming in contact with virus.

Exiting the room:

-Hose lines will be disinfected with bleach prior to leaving the room.

-All waste will be disinfected with bleach before leaving the room (includes media, dishes, and pipets).

-Outer gloves, shoes and the door handle will be sprayed with 70% ethanol prior to exiting the room.

-All gloves and lab coats will be removed upon exiting the room.

-Personnel will be required to wash their hands with waterless soap followed by a thorough handwashing with soap in the sink just outside the room.