THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR LAURA HERTEL
SIGNATURE Laura Hertel
DEPARTMENT MICROBIOLOGY & IMMUNOLOGY
ADDRESS HEALTH SCIENCE ADDITION HSA 320
PHONE NUMBER 84009
EMAIL lhertel@uwo.ca

Location of experimental work to be carried out: Building(s) HSA Room(s) 316, 310

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR / NSERC / J.P. BICKELL FOUNDATION
GRANT TITLE(S):

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

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

MATTHEW MILLER	DANIELLE VILLENEUVE
KARLY LAVERTY-LAMBERT	
WENDY FURLONG	
MICHAEL COHEN	
NICOLE BINDSEIL	

1.0 Microorganisms

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

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

Do you use microorganisms that require a permit from the CFIA? ○ YES ☒ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____
Please describe the risk (if any) of escape and how this will be mitigated:

_____
_____
_____

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

_____
_____
_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
E. coli DH5α	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	0.5	Lab Stock	☒ 1 ○ 2 ○ 3
Cytomegalovirus (Human)	☒ Yes ○ No	○ Yes ☒ No	○ Yes ☒ No	0.8	E.S. Mocarski (Stanford U.)	○ 1 ☒ 2 ○ 3
VSV-G pseudotyped retroviruses	○ Yes ☒ No	○ Yes ☒ No	○ Yes ☒ No	50 ml	E.S. Mocarski	○ 1 ☒ 2 ○ 3
	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No			○ 1 ○ 2 ○ 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒ Yes ○ No	Passaging of lab stocks originally from E.S. Mocarski	Not applicable
Rodent	☒ Yes ○ No	House embryo fibroblasts from Dr. R. Evans, U of Colorado	✓
Non-human primate	○ Yes ☒ No		
Other (specify)	○ Yes ☒ No		

PRIMARY CELLS:
Human Foreskin Fibroblasts ——— Stocks from E.S. Mocarski
Human Dermal Fibroblasts (lacrimal)
Human Dermal Fibroblasts (Giant Axonal Neuropathy patients) } Repository for Human Mutant Cell Strains - Montreal Children's Hospital
Human CD34+ progenitor cells
Human CD14+ monocytes } Cord blood - St. John's Hospital London

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

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	HeLa - HEK 293T ——— HeLaS3	E.S. Mocarski (Stanford U) D. Litchfield (UWO)
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 HC or CFIA containment level required ○ 1 ☒ 2 ○ 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Cord blood St. John's Hospital	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α	pcDNA 3.1 myc/his LNCX & derivatives p3xFLAG	Invitrogen E.S. Mocarski Sigma	Human CMV ORFs: US9, cORF29, cORF16 US7, UL120, UL109	Cells are used to identify the intracellular localization of transfected proteins by immunofluorescence analyses or to assess expression levels by western Blot. No stable cell lines are made. Cells are discarded after use or are fixed for immunofluorescence or are lysed.
	LNCX-GFP LNCX-3xmyc	Lab made Lab made	Human genes: * Cathepsin C * Sperm Associated Antigen 4 * Nucleolin	
	pCMV tat pJK3 pL-VSVG	E.S. Mocarski		
	pBluescript SKII	Stratagene		

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

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
VSV-G pseudotyped retroviruses	LNCX, pCMV tat, pJL3, pL VSVG	E.S. Haarsma	As per 4.2	As per 4.2 This method is used to transfect cells like fibroblasts

* Please attach a Material Safety Data Sheet or equivalent. N/A See attached refs.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ☒ YES ○ NO

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

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

5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? _____

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

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

6.0 Animal Experiments

6.1 Will live animals be used? ○ YES ☒ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals ○ YES, specify: _____ ○ NO

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ☒ NO If no, please proceed to Section 9.0

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

8.3 What is the LD_{50} (specify species) of the toxin_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ☒ NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

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

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O 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 plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

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

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____
If no, please proceed to Section 12.0 ☒ NO

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

11.3 Has an import permit been obtained from CFIA for animal or plan pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE Carlie Geitel

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

13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 X 2 O 3

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

X YES, permit # if on-campus BIO - UWO - 0125
O NO
O NOT REQUIRED

14.0 Procedures to be Followed

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

SIGNATURE Jamie Fritel Date: March 29, 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

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

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

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

Special Conditions of Approval:

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

Laura Hertel – Research projects requiring use of biohazards

My research focuses on:

1) The identification of the cis- and trans-acting elements that mediate maintenance of the CMV genome during latency in self-renewing and in differentiating CD34+ cells. Biohazards: CD34+ cells harvesting from cord blood samples; infection of CD34+ cells with CMV; cloning of selected CMV open reading frames (ORF) by reverse-transcription PCR of cDNA derived from CMV-infected human fibroblasts (HF); insertion of the cDNA coding for these ORFs into mammalian expression vectors (LNCX, LGFP, L3myc, p3xFLAG, pcDNA3.1myc/his), in frame with tags (3xFLAG, 3xmyc) or fused to GFP; expression of these ORF in HEK 293T cells, HeLa or in HF by cell transfection; if efficiency of transfection in HF is too low, proceed with cell transduction using VSV-G pseudotyped retroviruses (see below).

2) The identification of the binding partners of the CMV protein US9. Two interacting proteins were identified in yeast two-hybrid screens performed in Dr. Joe Mymryk's lab (note: no yeasts are or were grown in my lab). Verification of interactions and determination of their functional role is in progress. Biohazards: cloning of the two partners, Cathepsin C and Sperm associated antigen 4 into mammalian expression vectors as above. Expression of these genes in HEK 293T cells, HeLa, and HF, alone or in combination with US9. Neither gene is known to be an oncogene, nor will stable cell lines expressing these genes be produced.

3) The identification of the binding partners of the CMV protein cORF29. A yeast two-hybrid screen will be performed in Dr. Joe Mymryk's lab and selected partners will be tested for their binding to cORF29. Biohazards: eventual cloning of the cellular partners into mammalian expression vectors; expression of cORF29 and of the binding partners in HEK 293T cells, HeLa, and HF.

4) The identification and functional characterization of CMV proteins localizing to mitochondria. Biohazards: cloning and expression of selected CMV ORF into HEK 293T cells, HeLa and HF to assess their subcellular localization and the effect of domain deletions on their trafficking by immunofluorescence analysis.

5) The identification of the cellular genes transcriptionally altered during maturation of two types of dendritic cells, the Langerhans cells (LC) and the monocyte-derived dendritic cells (MDDC), derived *in vitro* from cord blood CD34+ and CD14+ progenitors. Biohazards: CD34+ and CD14+ cells harvesting from cord blood samples; reverse-transcription PCR analyses of cDNA derived from immature and mature LC and from immature and mature MDDC.

6) The identification of the mechanisms used by CMV to enter into mature and immature LC and into mature and immature MDDC. Biohazards: CD34+ and CD14+ cells harvesting from cord blood samples; infection of LC and MDDC with CMV and tracking of viral particles by immunofluorescence staining analyses.

Cloning of specific genes will be done by reverse-transcription PCR. cDNAs will be inserted in mammalian expression vectors, which will be subsequently transformed into the *E.coli* strain DH5-alpha. Vectors containing the cloned genes will be transfected in cell lines (HEK 293T and HeLa), and in primary cells (HF) for expression analyses. Transfection efficiency of primary cells is usually low. To ensure expression of the cloned gene in at least 80% of these cells, we will produce and use VSV-G pseudotyped retroviruses generated following the protocol published by Bartz S.R. et al, Methods, 12:337-342, 1997 (paper attached). Using this method, the cDNA of interest is cloned into the expression vector pLNCX under the control of the CMV promoter. Pseudotyped retroviral particles containing the expression vector are then produced after transfection of HEK 293T cells with LNCX, pL-VSV-G, pJK3 (MLV gag/pol expression plasmid) and pCMV-tat. The CMV-tat plasmid is used to produce HIV-1 tat to drive expression of pJK3 and pL-VSV-G, whose expression is under the control of the HIV-1 LTR.
Note that the pseudotyped retroviruses produced with this method are not replication competent.

Cell Biology

ATCC® Number:	**CCL-2™** · Order this Item	Price:	**$256.00**
Designations:	HeLa	Depositors:	WF Scherer
Biosafety Level:	2 [CELLS CONTAIN PAPOVAVIRUS]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	PHOTO
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.		
	Related Cell Culture Products		
Applications:	transfection host ([21491] technology from amaxa Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]		
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3		
Reverse Transcript:	negative		
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18		
Cytogenetic Analysis:	Modal number = 82; range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of Ml, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm		

	of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 derivative:ATCC CCL-2.1 derivative:ATCC CCL-2.2 derivative:ATCC CCL-2.3
Bioreactive Factors:	**Growth Factors:** T cell growth factor (TCGF)
	21447: American Public Health Association. Compendium of methods for the

Cell Biology

ATCC® Number:	**CCL-2.2™** Order this Item	Price:	**$268.00**
Designations:	HeLa S3	Depositors:	TT Puck
Biosafety Level:	2 [Cells contain human papilloma virus (HPV-18)]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent epithelial
Organism:	*Homo sapiens* (human)	Morphology:	
Source:	**Organ:** cervix **Disease:** adenocarcinoma		
Cellular Products:	keratin		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Isolation:	**Isolation date:** 1955
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
Virus Susceptibility:	poliovirus 1, 2, 3; vesicular stomatitis (Indiana); encephalomyocarditis; adenovirus 5
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18
Cytogenetic Analysis:	A medium-sized metacentric marker is present in 100% of the cells. HeLa Markers: One copy of M1, one copy of M2, two copies of M3, and one copy of M4.
Isoenzymes:	G6PD, A
Age:	31 years
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y

HeLa S3 is a clonal derivative of the parent HeLa line (see ATCC CCL-2). S3 was cloned in 1955 by T.T. Puck, P.I. Marcus, and S.J. Cieciura. [22814]
The HeLa S3 clone has been very useful in the clonal analysis of mammalian cell populations relating to chromosomal variation, cell nutrition, and plaque-forming

ability.
This line can be adapted to grow in suspension. [25952]
The cells are positive for keratin by immunoperoxidase staining.

Comments: A culture at approximately passage 400 was submitted to the American Type Culture Collection in February, 1972.
HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. [23180]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated F-12K Medium, Catalog No. 30-2004. 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
Protocol:

Subculturing:

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 1:10 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 30-2004
recommended serum:ATCC 30-2020

22263: Chen TR. Re-evaluation of HeLa, HeLa S3, and HEp-2 karyotypes. Cytogenet. Cell Genet. 48: 19-24, 1988. PubMed: 3180844
22766: Boshart M, et al. A new type of papillomavirus DNA, its presence in genital cancer biopsies and in cell lines derived from cervical cancer. EMBO J. 3: 1151-1157, 1984. PubMed: 6329740
22814: Puck TT, et al. Clonal growth of mammalian cells in vitro; growth characteristics of colonies from single HeLa cells with and without a feeder layer. J. Exp. Med. 103: 273-283, 1956. PubMed: 13286432
23180: Yee C, et al. Presence and expression of human papillomavirus sequences in human cervical carcinoma cell lines. Am. J. Pathol. 119: 361-366, 1985. PubMed: 2990217
25929: Puck TT, Marcus PI. A rapid method for viable cell titration and clone

Cell Biology

ATCC® Number:	**CRL-11268™** Order this Item	Price:	**$264.00**
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.		

This material is cited in a U.S. and/or other Patent or Patent Application, and may not be used to infringe on the patent claims. ATCC is required to inform the Patent Depositor of the party to which the material was furnished.

Related Cell Culture Products

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]
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 **Protocol:**
Subculturing:	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:	derivative:ATCC CRL-11269 recommended serum:ATCC 30-2020 Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
References:	45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361 57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999 57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001 57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

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Production of High-Titer Human Immunodeficiency Virus Type 1 Pseudotyped with Vesicular Stomatitis Virus Glycoprotein

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We describe a method for the production of high-titer stocks of human immunodeficiency virus type 1 (HIV-1) pseudotyped with vesicular stomatitis virus envelope glycoprotein (VSV G). VSV G pseudotypes provide several advantages over other retroviral envelope proteins. The VSV G envelope is mechanically stable, enabling ultracentrifugal concentration of virions to high titers, and VSV G has a broad host range, enabling infection of many mammalian and nonmammalian cell types. VSV G pseudotypes of HIV-1 are useful for the study of HIV infection and replication kinetics and for the study of the function of specific viral proteins. We describe applications for the study of HIV-1 using VSV G pseudotypes. Additionally, we describe a method for pseudotyping retroviral vectors with VSV G. The same advantages of VSV G pseudotypes of HIV-1 apply to retroviral vectors; VSV G pseudotyped retroviral vectors may be used to introduce genes of interest into a wide variety of cell lines.

Pseudotyped human immunodeficiency virus type 1 (HIV-1) virions have been, and continue to be, important in the study of HIV-1 replication. Likewise, cell lines stably expressing HIV-1 gene products have provided a wealth of knowledge of HIV-1 biology. HIV-1 pseudotypes are virions containing all the proteins and core structure of HIV-1 but packaged inside the envelope protein of a different virus. Pseudotyped HIV-1 particles, and pseudotyped retroviral vectors used for establishing stable cell lines, have traditionally been produced using the amphotropic envelope of the Moloney murine leukemia virus (MLV). However, the MLV envelope has a limited host range, and like the native HIV-1 envelope protein, gp120, the amphotropic envelope is not stable under ultracentrifugation, preventing concentration of virions. These limitations can be overcome using the envelope glycoprotein of vesicular stomatitis virus (VSV). The VSV envelope glycoprotein (VSV G) is also able to pseudotype retroviral particles, and VSV G pseudotypes may be concentrated because unlike the native retroviral envelope proteins, the VSV G association with the virion is stable during ultracentrifugation. In addition, VSV G has a broad host range, permitting the entry of virions into most mammalian as well as nonmammalian cell types (1, 2).

We routinely use the VSV G protein to produce high-titer pseudotyped HIV-1 stocks by transient cotransfection of a plasmid containing an *env*-deleted provirus and a VSV G expression plasmid. We also produce VSV G pseudotypes from proviral constructs containing mutations or deletions of various HIV-1 genes to generate a homogenous virus population carrying the introduced mutation. Wild-type and mutant virions are useful for many experimental applications requiring a single round of infection. With the high-titer viral stocks produced, 100% infection of some cell types is possible. Not having to remove uninfected cells from a population facilitates biochemical analysis of HIV-1 replication and genetic analysis of HIV-1 mutants. Further analysis of gene function is possible by generating cell lines stably expressing a gene, or genes, of interest using the VSV G pseudotypes. VSV G also overcomes the problem of tropism encountered with native HIV-1 envelopes because it promotes efficient infection of both of the major targets of HIV-1, T-cells and macrophages. The broad host range and high-titer stocks of VSV G pseudotypes provide flexibility for the HIV-1 researcher.

MATERIALS

Plasmids

Plasmids are prepared using Qiagen (Chatsworth, CA) midi or maxi preps. The plasmid pL-VSV G, which

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contains the VSV G gene cloned downstream of the HIV-1 long terminal repeat (LTR), is used to obtain high-level expression of VSV G and is cotransfected with plasmid pBru3Δenv containing the HIV-1_{Lai} provirus, which has a deleted *env* gene but is otherwise wild type (3–5).

For the production of pseudotype virions containing retroviral vectors, the following plasmids are used: pL-VSV G; pJK3 (MLV gag pol expression plasmid); pBabe-puro, pLxSN, or pLxSH; and pCMV-tat (6, 7). The CMV-tat plasmid is used to produce HIV-1 Tat to drive expression of pJK3 and pL-VSV G because their expression is under control of the HIV-1 LTR. The gene one wishes to study is cloned into pBabe-puro, pLxSN, or pLxSH, allowing for selection of stable cell lines using the drugs puromycin, neomycin, or hygromycin, respectively.

Solutions for Transfections

All solutions used for transfection should be filter-sterilized, stored at −20°C, and thawed to room temperature before use. The following solutions were used: 2× BES-buffered saline pH 6.95 (50 mM *N,N*-Bis BES [free acid, not the sodium salt; Sigma Catalog No. B-6266], 280 mM NaCl, 1.5 mM Na_2HPO_4); 0.25 M $CaCl_2$.

Cell Lines

The 293T human embryonic kidney cell line is available from American Type Culture Collection. The MAGI cell line, which is used for determining viral titers, can be obtained from the NIH AIDS Repository. The cells are grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum.

Equipment

A Biosafety Level 3 (BL3) facility, equipped with an ultracentrifuge and standard tissue culture equipment, is necessary.

METHOD

An overview of the method is diagrammed in Fig. 1.

Day 0: Plate Cells

One day before transfection, plate 293T cells at 4.5 × 10^5 to 6 × 10^5 cells per well in a 6-well plate, with 2 ml of medium per well.

Day 1: Transfect Cells Using Calcium Phosphate Precipitation

Transfect cells when they have reached approximately 70% confluence. If cells are too confluent, transfection efficiency is decreased, and if cells are underconfluent, virus yields are substantially decreased. We use the calcium phosphate precipitation method for transfection (8); other methods resulting in high transfection efficiency would presumably work as well.

The quantities given are for a single well of a 6-well plate; scale up accordingly for the number of wells to be transfected. Use 2 μg of plasmid encoding the HIV provirus with an *env* deletion (pBru3Δenv) and 1 μg of plasmid encoding VSV G (pL-VSVG). Add the appropriate volume of plasmid DNA to 175 μl of 0.25 M $CaCl_2$, vortex, and then add 175 μl of 2× BBS. Vortex and incubate at room temperature for 10–15 min. Add 350 μl of solution, dropwise, to each well. Rock plates gently. Examine the wells with a microscope, as a precipitate is often present after transfection. After addition of transfection mixture, these cells must be removed to a BL3 facility.

The same method is used for pseudotyping MLV virions containing retroviral vectors except that 1 μg of pJK3, 2 μg of pBabe-puro (or pLxSN, or pLxSH), 1 μg of pL-VSV G, and 0.1 μg of pCMV-tat are used per well. Production of retroviral vectors may be conducted under standard BL1/2 conditions.

Note: All subsequent steps for the production of pseudotyped HIV-1 virions must be performed in a BL3 facility using appropriate BL3 safety practices.

Day 2: Wash Cells

One day posttransfection, aspirate medium and carefully wash wells one time with 1 ml phosphate-buffered saline (PBS) and gently add back 2 ml fresh medium. It is important to examine the cells for the effects of VSV G. If cells look normal, healthy, and undamaged, collect virus the following day. If cells are fusing and coming off the plate due to expression of VSV G, wait 6–8 h and collect the supernatant that day. For increased yields, even if cells look healthy, supernatant may be collected on Day 2 in the evening, after washing in the morning, and combined with supernatant collected on Day 3.

Day 3: Collect and Concentrate Virus

Collect medium from cells and transfer to a conical tube. Spin at approximately 700*g* for 10 min in a swinging bucket centrifuge to pellet cells and debris. Carefully remove the cell-free supernatant to an appropriate size ultracentrifuge tube. (*Note:* Depending on the viral titer desired, concentration of the virus may not be necessary.) The Beckman SW41ti rotor holds tubes that will contain the total volume of a 6-well plate. Smaller or larger volumes will require smaller or larger rotors. In addition to the SW41ti, we have successfully used the SW28 and the SW55 rotors. Regardless of the rotor/tube size, spin in ultracentrifuge at 23,000 rpm for 1.5–2 h. At the end of the spin, promptly remove tubes.

To resuspend the viral pellet, decant medium from each tube, and leave tubes inverted on absorbent paper

to remove as much medium as possible. Sometimes a translucent pellet is visible, but often is not. Add the desired volume of cell culture medium or PBS to the pelleted virus. Typically we aim for a 100-fold concentration, so we add 1/100 of the starting volume. Seal tubes (with Parafilm or by returning the tubes to the bucket and sealing the lids) and store at 4°C for 1 h. Using a micropipette, pipette the medium up and down and wash around the bottom sides of the tube. We store the virus stocks in small aliquots, in screw-cap microcentrifuge tubes at −80°C. We have stored virus stocks for 12 months or longer with no apparent loss of viral titer. Others have reported that VSV G pseudotypes are stable to multiple freeze–thaw cycles (1), and we have observed no decrease in VSV G pseudotyped HIV-1 titers after 3 freeze–thaw cycles.

For viruses that give low yields, pool multiple first-round concentrations for a second round of concentration.

Titering of Viral Stocks

We use the MAGI cell line, which is available from the NIH AIDS Repository, for determining the titer

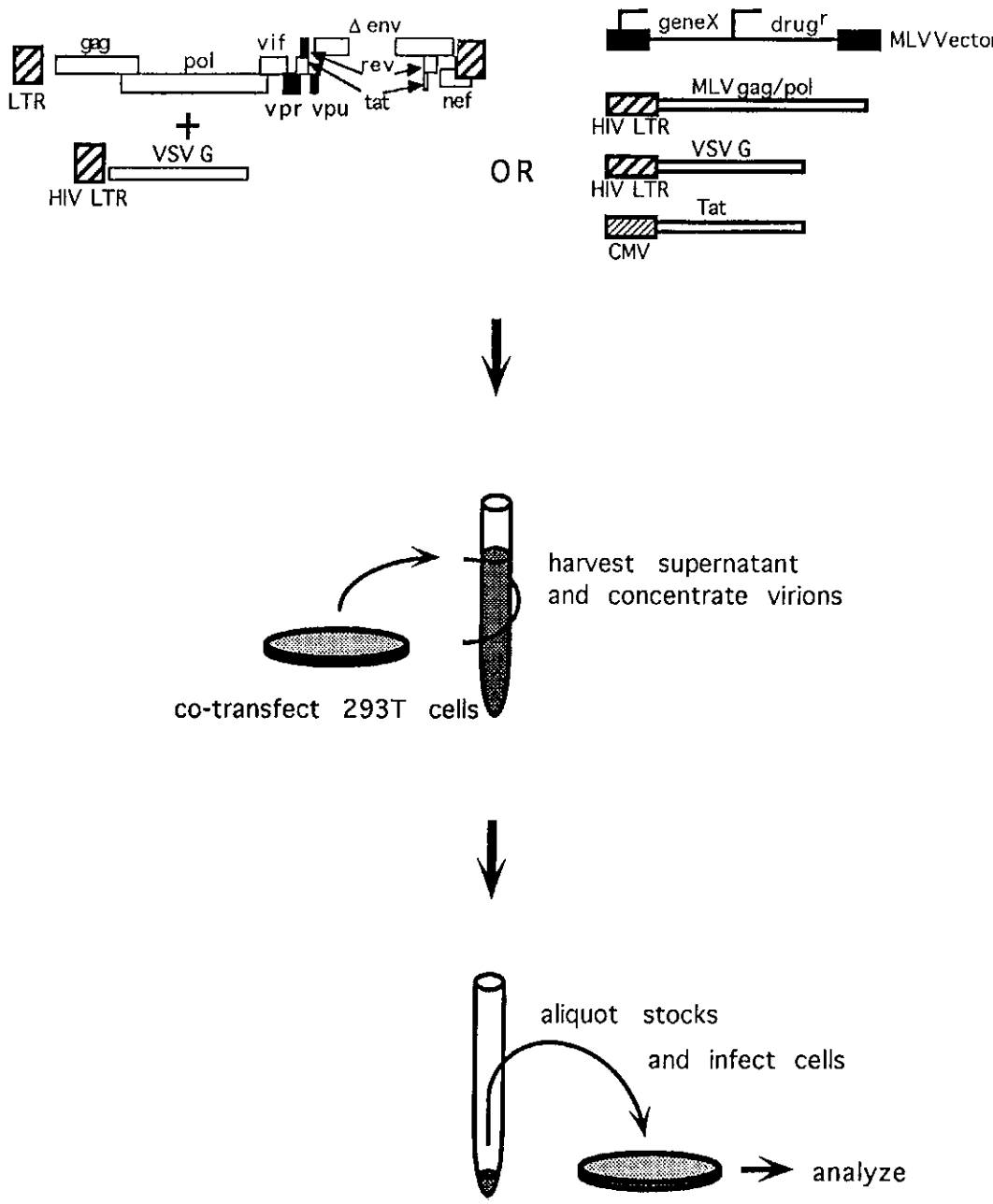

FIG. 1. Schematic overview of the production of high-titered VSV G pseudotyped retroviral stocks.

of VSV G pseudotyped HIV-1 viral stocks. A detailed method on the use of MAGI cells for determining viral titers has been described elsewhere (9).

Applications and Infection of Cell Lines

We have infected a number of different cell types, primary cells and transformed cell lines, with VSV G pseudotyped HIV-1 and pseudotyped retroviral vectors. For cells infected with the retroviral vectors, the appropriate selective drug is added 1 day postinfection.

The following protocol describes the use of VSV G pseudotypes of HIV-1 to study the events occurring in a single round of infection in Jurkat cells, a human T-cell line that grows as a suspension culture. This protocol may also be followed as a general method to infect suspension cell cultures using these pseudotypes.

1. Pellet 2×10^6 Jurkat cells and aspirate all but approximately 50–100 μl of the medium.
2. Add virus at a multiplicity of infection (m.o.i.) of 10. For example, if the concentration of the viral stock is 2×10^9 infectious units/ml (IU/ml), then add 10 μl of virus. Add DEAE–dextran to a final concentration of 10 μg/ml (prepare a stock of 100 or 200 μg/ml), and incubate the cells for 2 h at 37°C.
3. After the 2-h incubation, wash the cells one time with PBS or directly add medium and place the cells in culture. We typically resuspend the cells to a final concentration of 5×10^5 cells/ml.
4. At selected hours or days after infection, analyze the infected cells. Such analyses may include events in the HIV-1 life cycle and the effect(s) of HIV-1 replication, or gene products, on cellular events. Depending on the application, it may or may not be necessary to quantify the percentage of infected cells. However, to determine the percentage of cells infected we use flow cytometry and the anti-gag p24 monoclonal antibody (mAb) KC57 (Coulter Immunology) conjugated with either fluorescein isothiocyanate or phycoerythrin. Briefly, pellet the cells by centrifugation, resuspend in 1% paraformaldehyde in PBS, and incubate at least 0.5 h (or up to 24 h) at 4°C. Pellet the cells and permeabilize by resuspending the cells in 0.5% Tween 20 in PBS for 5–10 min. Pellet the cells, resuspend in 200 μl of PBS, add the appropriate amount of antibody, and incubate for 0.5 h at room temperature. At the end of the incubation, wash the cells with PBS, pellet, resuspend in 0.5 ml of PBS and then analyze by flow cytometry. Typical results displayed in Fig. 2.

Adherent cells can be infected while still attached to plastic or as a suspension culture. To infect adherent cells in suspension, remove cells from plastic using a nonenzymatic cell dissociation solution and then infect the cells as described above.

The following protocol describes the use of VSV G pseudotyped HIV-1 virus carrying a deletion in the *env* gene and frameshift mutation in *vpr* to create a cell line chronically producing noninfectious HIV-1 particles. This also serves as a general protocol for infection of adherent cells using VSV-G pseudotypes.

1. One day prior to infection, plate HeLa cells at 0.8×10^5 per well in a 12-well plate or at 2×10^5 per well in a 6-well plate.
2. On the day of infection, aspirate medium and infect at a m.o.i. of 10 by adding back medium and virus to a final volume of 300 or 600 μl, for a 12-well or 6-well plate, respectively. Add DEAE–dextran to a final concentration of 20 μg/ml. Incubate for 2 h at 37°C. The plate can be placed on a rocking platform during the incubation, but this may not be necessary.
3. Aspirate the virus-containing medium from the cells, wash once with PBS, and then add back 1 or 2 ml of medium for a 12- or 6-well plate, respectively. Alternatively, the infection may be stopped by adding fresh medium without removing the virus-containing medium.
4. One day postinfection, trypsinize cells and replate into 6-well plates by limiting dilution. After 3–4 days, use cloning rings to pick single-cell clones and replate. Expand the clones until 30–50% confluent and assay cell-free medium for p24 production by ELISA (we use Coulter Immunology).

RESULTS

Using the described method, we routinely produce high-titer VSV G pseudotyped viral stocks. Typically, a yield of approximately 10^7 IU/ml is obtained for VSV G pseudotyped HIV-1 without concentration. The titer is easily increased to 10^9 IU/ml or more following concentration by ultracentrifugation. HIV-1 proviruses that have mutations in genes affecting viral replication will usually result in lower viral titers. The titers of the VSV G pseudotyped MLV retroviral vectors average about 10^4 to 10^6 colony-forming units/ml. These stocks can also be concentrated.

We observe variability in infection efficiency with the pseudotyped HIV-1 stocks using the same m.o.i. (as titered on MAGI cells) on different cell lines. This is true even when the same cell lineage is infected, for example, human T cells. Infecting Jurkat or MT-4 cells at a m.o.i. of 10 results in infection of essentially 100% of the cells (Fig. 2; Ref. (3)). However, infecting primary CD4 T cells or the H9 cell line at a m.o.i. of 10 results in infection of between 10 and 30% or of approximately 60% of cells, respectively (Fig. 2 and data not shown). In addition to T-cells, we have used the VSV G pseudotyped stocks to infect a number of

other cell types, including primary human macrophages, HeLa cells (cervical), A549 cells (lung), and AT cells (fibroblast).

We suggest several potential causes and remedies if one obtains lower than expected viral titers following the described method. The most likely cause of low titers is low transfection efficiency. We recommend testing transfection solutions using a β-galactosidase (β-gal) expression plasmid and then staining for β-gal activity, or a similar reporter plasmid and assay, before using solutions for production of virus. The pH of the 2× BBS solution is crucial, and this reagent should be prepared with care. Typically a precipitate is seen in the wells either immediately or a few hours after transfection. At 1 to 2 days after transfection, extensive cell damage from membrane fusion due to VSV G should be observed in the wells. If no precipitate or cell damage is apparent, then the quality of the transfection reagents should be evaluated.

Another possibility for observably low titers may be low infection efficiency rather than low viral production. We have not determined if other cationic agents such as polybrene would yield better infection efficiencies than DEAE. However, the method detailed for the infection of Jurkat results in infection of nearly 100% of the cells. It has been reported that different cationic agents can dramatically change the infection efficiency of VSV G (1).

Viral titers may also be improved by collecting medium every 8 h, storing it at 4°C, and pooling the collected medium for concentration by ultracentrifugation. Additionally, low titers are obtained with some mutant proviral constructs that replicate inefficiently. To obtain high-titer pseudotyped stocks with these viruses, multiple rounds of concentration must be employed.

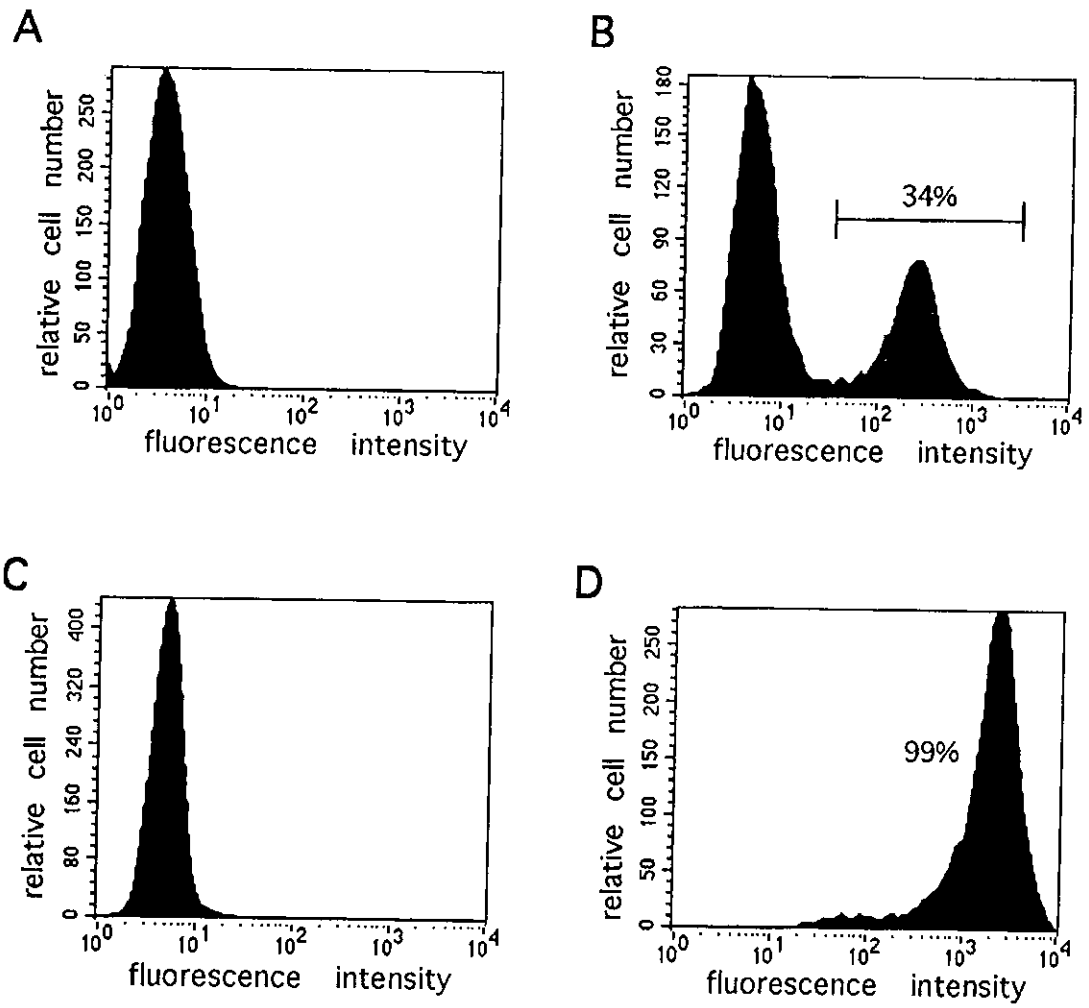

FIG. 2. Analysis for the percentage of cells infected by VSV G pseudotyped HIV-1. Primary CD4 cells (A and B) or the human T cell line MT-4 (C and D) were either mock-infected (A and C) or infected with VSV G pseudotyped HIV-1 (B and D) at a m.o.i. of 10. Two days postinfection cells were fixed, permeabilized, and then stained with the mAb KC57 conjugated to either FITC or PE (Coulter Immunology). The cells were analyzed by flow cytometry. The percentage of cells infected is indicated in B and D.

CONCLUDING REMARKS

We have described a simple method for production of high-titer HIV-1 pseudotypes using VSV G and provided two protocols as examples for using the pseudotypes to study HIV-1. High titers and a broad host range of VSV G pseudotyped HIV-1 and retroviral vectors make this a potent method for studying HIV-1 and other areas of cell and molecular biology.

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