**THE UNIVERSITY OF WESTERN ONTARIO**
**BIOHAZARDOUS AGENTS REGISTRY FORM**
**Approved Biohazards Subcommittee: July 25, 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 are 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. The form must also be completed if any work is proposed involves plants 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 required 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 (Stevenson-Lawson Building, Room 295) 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 Rodney DeKoter
SIGNATURE Rodney DeKoter
DEPARTMENT Microbiology and Immunology
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PHONE NUMBER
EMAIL rdekoter@att.net

Location of experimental work to be carried out: Building(s) Dental Sciences Room(s) 3003

*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
GRANT TITLE(S): Regulation of myeloid versus lymphoid cell fate by PU.1 (pending)
Functions of related Ets transcription factors in B cells (pending)

**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:

TBA

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

## Summary of Biohazards – Rodney DeKoter, Ph.D.

Our laboratory studies gene regulation in the immune system. Our goal is to learn the mechanism(s) of how genes are turned on or off by proteins called transcription factors. To do these experiments, we rely on two experimental approaches. First, we use transgenic or gene targeting technology to modify genes in mice. Our laboratory maintains a number of lines of mice in which genes encoding transcription factors have been genetically modified. Second, we grow primary or transformed cells in culture to study gene expression. We use standard recombinant DNA technology to modify genes in plasmid vectors. We also use replication-incompetent retroviral vectors to transfer genes into cultured cells. Our laboratory does not work with retroviral vectors capable of replicating in culture or in live organisms. Our laboratory does not work with infectious microorganisms.

Summary – Biohazards in our laboratory include

- Recombinant DNA technology (using plasmids grown in E. coli)
- Genetically modified mice
- Replication-incompetent retroviral vectors

## 1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (Including but not limited to viruses, prions, parasites, bacteria)? ⊘YES O NO

If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O 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 |
|---|---|---|---|---|---|---|
| Ecotropic Retrovirus | O Yes ⊘No | O Yes ⊘No | O Yes ⊘No | N/A | N/A | O1 ⊘2 O3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O1 O2 O3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O1 O2 O3 |
| | O Yes O No | O Yes O No | O Yes O No | | | O1 O2 O3 |

*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 O 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 O No | BM, Spleen, thymus | Not applicable |
| Rodent | ⊘Yes O No | BM, Spleen, thymus | In progress |
| Non-human primate | O Yes ⊘No | | |
| Other (specify) | O Yes ⊘No | | |

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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 O No | Various | ATCC |
| Rodent | ✓ Yes O No | Various | ATCC, Mice |
| Non-human primate | O Yes ✓ No | | |
| Other (specify) | O 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 O2 O3

**3.0 Use of Human Source Materials**

3.1 Does your work involve the use of human source materials? ✓YES O 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 | | O Yes ✓ No | | O1 ✓2 O3 |
| Human Blood (fraction) or other Body Fluid | | O Yes O No | | O1 O2 O3 |
| Human Organs or Tissues (unpreserved) | | O Yes O No | | O1 O2 O3 |
| Human Organs or Tissues (preserved) | | O Yes O No | | O1 O2 O3 |

**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 O NO If no, please proceed to Section 5.0

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

| Bacteria Used for Cloning * | Plasmid(s) * | Source of Plasmid | Gene Transfected | Describe the change that results |
|---|---|---|---|---|
| E. coli. | Various | Various | Various | Various |

✓ * 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? O YES, complete table below O NO

| Virus Used for Transduction * | Vector(s) * | Source of Vector | Gene Transfected | Describe the change that results |
|---|---|---|---|---|
| Ecotropic Retrovirus | MIGR1 | Clontech | Various | Various |

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ⓘ YES O NO

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

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

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

**6.0 Animal Experiments**

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

6.2 Name of animal species to be used Mouse

6.3 AUS protocol # In progress

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

Retroviral-infected cells will be transplanted into mice. Virus will be replication-incompetent, non-infectious, and will not be directly given to animals.

*** 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:

_____
_____
_____

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

**10.0 Plants Requiring CFIA Permits**

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ 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 the agent be imported? O YES, please give country of origin_____ ⊘ NO
If no, please proceed to Section 10.0

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

11.3 Has an import permit been obtained from CFIA for animal 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 Safely
- 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 Rodney Devitt

*** 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 ☑ 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?
O YES, permit # if on-campus_____
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 have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE Rodney DeKoter Date: 1-13-2009

**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***

# Retroviral Expression Systems

Facts & FAQs

## Why use a retroviral expression system?

Expression systems based on retroviral gene delivery are generally faster, more reliable, and have broader utility than standard plasmid-based systems. These benefits are derived from aspects of retroviral biology.

- **Because retroviral infection is highly efficient, you can often work with heterogeneous, selected populations of cells and save the time and effort required for cloning cell lines.**
- **Stable integration of the virus into the host genome results in reliably heritable expression, without variability or loss of expression.**
- **Retroviruses preferentially integrate into actively transcribed regions of the genome and yield stable, highly expressing cell lines with no internal rearrangement of the gene of interest.**
- **Retroviral infection is one of the easiest and most reliable lab techniques—you simply apply virus-containing media to dividing target cells**

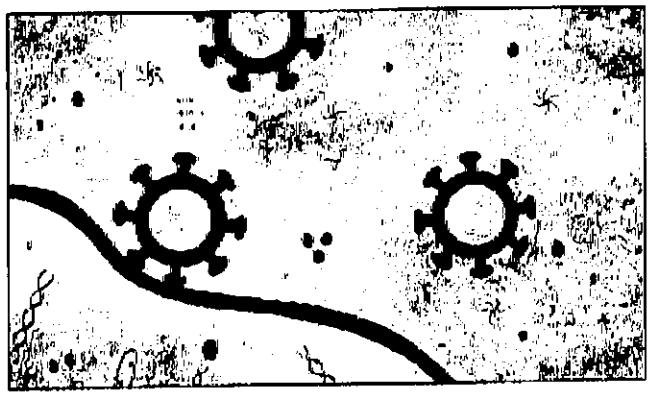
Retroviral infection specificity is determined by the envelope proteins of the viral particle that recognize specific receptors on the cell surface. A broad range of cell types can be readily infected depending on the packaging cell line used. Any mitotically dividing cell can be effectively infected—including primary cultures, embryonic stem cells and cell lines that are difficult to transfect.

## I'm concerned about safety. What are the risks?

The National Institute of Health and the Center for Disease Control have designated retroviral vectors such as those from Moloney murine leukemia virus (MoMuLV) as Biosafety Level 2. Virus produced from amphotropic and dualtropic packaging cell lines is capable of infecting human cells. However, depending on your retroviral insert, the recombinant viruses produced could be potentially hazardous. Vectors similar to those offered by Clontech have been approved for human gene therapy trials, attesting to their ability to express cloned genes *in vivo*. Due caution should always be exercised in the production and handling of any recombinant retrovirus.

## What type of facilities are needed?

The following is a brief description of a Biosafety Level 2 tissue culture facility. It is neither detailed nor complete. For more information, please visit the NIH website at www.niehs.nih.gov/odhsb/biosafe/bmbl/bmb-1.htm.



Practices:
- Perform work in a limited access area
- Post biohazard warning signs
- Minimize production of aerosols
- Decontaminate potentially infectious wastes before disposal
- Take precautions with sharps

Safety Equipment:
- Biological safety cabinet, (a laminar flow hood with a HEPA microfilter)
- Protective laboratory coats, face protection, double gloves

Facilities:
- Autoclave for decontamination of wastes
- Unrecirculated exhaust air
- Chemical disinfectants available for spills

**Clontech Laboratories, Inc.**
www.clontech.com

| United States/Canada | Europe | Asia Pacific | Latin America/Caribbean |
|---|---|---|---|
| 800.662.2566 | 32.53.720.211 | 81.77.543.7247 | 01.800.662.2566 |



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# Retroviral Expression Systems FAQs

## Q How are retroviral vectors used to express recombinant genes?

**A** The overall strategy is diagrammed in Figure 1. Cloning your gene of interest into a retroviral expression vector is no different than cloning it into a plasmid vector. The construct is then transfected into the packaging cell line using $CaPO_4$, electroporation, or any standard transfection method. Once inside the cell, the retroviral vector DNA is transcribed either in a transient fashion or from stably integrated copies. That RNA transcript migrates to the cytoplasm where the $\Psi^+$ signal is recognized by viral proteins produced by the packaging cell and packaged into infectious virus particles. The transcripts contain all elements between the 5' and 3' LTRs. All of the proteins needed for virus production, [*gag* (structural proteins), *pol* (integrases, reverse transcriptase), *env* (envelope glycoproteins)], are stably maintained in the genome of the packaging cell line. However, the virus particles are incapable of replicating themselves to produce further generations of virus because they do not include the *gag*, *env*, or *pol* genes. These particles are released into the culture medium which is then collected and used to infect the target cell line.

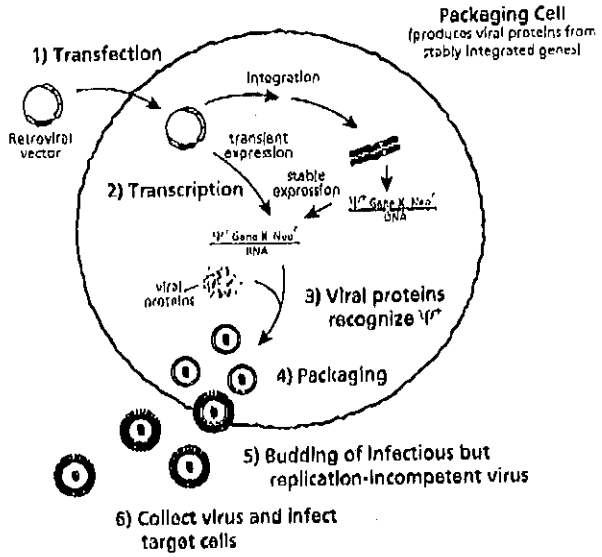


Figure 1. Packaging of retroviral particles with a packaging cell line. A retroviral vector containing at least the gene of interest, an antibiotic selection gene, and $\Psi^+$, the packaging signal necessary for retrovirus particle formation, can stably integrate or be transiently expressed. The packaging cell line provides the genes necessary for particle formation which have been deleted from the vector: *gag* (core structural proteins), *pol* (reverse transcriptase, integrase), and *env* (coat glycoproteins). Virus released from the packaging cell line contains the products of these genes (and is infectious), but lacks the genes themselves, thus preventing retroviral production from subsequently infected cell lines.

## Q What is the limit on the size of my DNA insert?

**A** Retroviruses efficiently package RNA that is less than 8–9 kb. The length of the transcript for packaging is determined as the distance from the 5' LTR to the 3' LTR in Clontech's retroviral expression vectors. RNA containing inserts up to 5 kb in size can be packaged efficiently without a reduction in viral titer.

**Table I: Host range of packaging cell lines expressing different envelopes**

| | Envelopes | | | |
|---|---|---|---|---|
| Target Cells | 10A1 (expressed by PT67) | Amphotropic | GALV | Ecotropic |
| Mouse | + | + | – | + |
| Rat | + | + | + | + |
| Hamster | + | +/– | + | – |
| Mink | + | + | + | – |
| Cat | + | + | + | – |
| Dog | + | + | + | – |
| Monkey | + | + | + | – |
| Human | + | + | + | – |

(+) = transduced; (–) = not transduced. Data courtesy of Miller & Chen, 1996.

## Q Do I need to include a poly A+ sequence when cloning my gene of interest into a retroviral expression vector?

**A** No, the native polyadenylation signal from the wild-type virus is contained in the 3' LTR of all retroviral expression vectors. This 3' LTR sequence is sufficient for polyadenylation of the transcribed gene of interest and is required for integration of the retrovirus into the genome.

# Retroviral Expression Systems FAQs...continued

## Q How do I choose which packaging cell line to use?

**A** The envelope protein made by the packaging cell line determines the range of infectivity (tropism) of the viral particles that the cell line produces. Viral envelopes are classified according to the receptor used to enter the host cell (Table I). Amphotropic viruses enter host cells through the amphotropic receptor, and ecotropic viruses utilize the ecotropic receptor. Dualtropic envelopes are able to utilize the amphotropic and the GALV receptor, increasing their host range.

Clontech's RetroPack™ PT67 Packaging Cell Line expresses a dualtropic envelope that recognizes receptors found on cells from mouse, rat, human, hamster, mink, cat, dog, and monkey. The *env* gene of RetroPack PT67 is derived from the 10A1 strain of murine leukemia virus which recognizes two widely expressed receptors, Glvr-1 (Pit-1) and Ram-2 (Pit-2). The presence of two potential viral receptors means that if one receptor is not abundantly expressed by a given species or cell type (as in the case of many rat cells, where Glvr-1 is not present at a significant level), the alternate receptor may still allow for efficient entry. Thus, the PT67 Cell Line not only produces virus with a broad host range, but the resultant virus may also contribute to greater infectivity for cell types that may have been resistant to infection by virus made from amphotropic packaging cell lines.

Clontech's EcoPack™-293 Cell Line (to be released early in 1999) is an ecotropic packaging cell line derived from easy-to-transfect 293 cells. This packaging cell line is ideal for fast generation of high-titer recombinant virus that can infect mouse and rat cells.

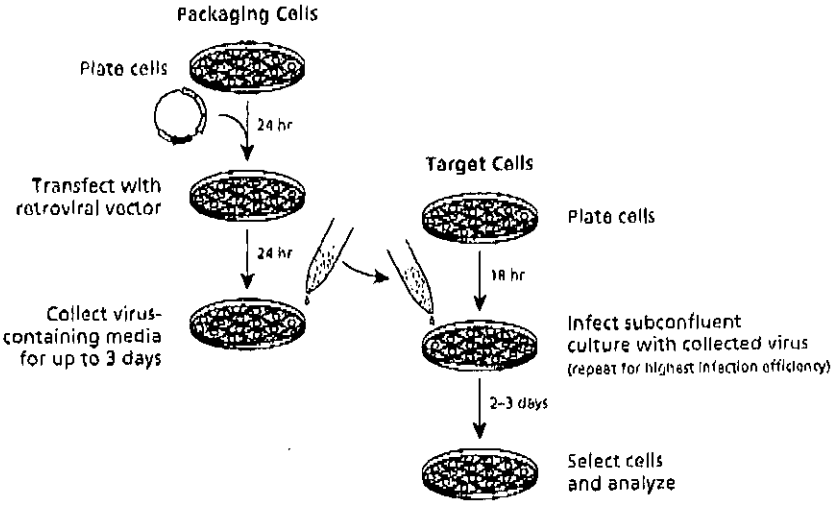


Figure 2. Establishing a retroviral expression system.

## Q Can primary cells be infected with retrovirus?

**A** Yes, as long as they are mitotically active. Most terminally differentiated cells (e.g., neurons and muscle cells) cannot be infected.

## Q How many of my cells should I expect to be infected?

**A** With a good viral titer (>$10^5$/ml), up to 90% of cells can be infected. Serial infections can be performed to increase infectivity up to nearly 100%. To determine the efficiency of infection, plate infected cells in parallel, with and without antibiotic, and compare the number of colonies after the selection is complete. To quickly determine the percent of infected cells, perform alkaline phosphatase staining of cells infected in parallel with virus derived from cells transfected with pLAPSN (expresses alkaline phosphatase).

## Q My retroviral vector DNA didn't cut as predicted and is difficult to grow. Why?

**A** Retroviral vectors recombine and rearrange more easily than other vectors because of the presence of direct repeat sequences in the LTRs. Try an alternate *E. coli* host strain designed to handle such vectors.

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# Retroviral Systems Bibliography


1. Bartek, J., Bartkova, J., Kyprianou, N., Lalani, E.-N., Staskova, Z., Shearer, M., Chang, S. & Taylor-Papadimitriou, J. (1991) Efficient immortalization of luminal epithelial cells from human mammary gland by introduction of simian virus 40 large tumor antigen with a recombinant retrovirus. *Proc. Natl. Acad. Sci. USA* 88:3520–3524.
2. Bender, M. A., Gelinas, R. E. & Miller, A. D. (1989) A majority of mice show long-term expression of a human b-globin gene after retrovirus transfer into hematopoietic stem cells. *Mol. Cell. Biol.* 9:1426–1434.
3. Brown, M., McCormack, M., Zinn, K. G., Farrell, M. P., Bikel, I. & Livingston, D. M. (1986) A recombinant murine retrovirus for simian virus 40 large T cDNA transforms mouse fibroblasts to anchorage-independent growth. *J. Virol.* 60:290–293.
4. Burns, J. C., Friedmann, T., Driever, W., Burrascano, M. & Yee, J. K. (1993) Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: Concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. *Proc. Natl. Acad. Sci.* 90:8033–8037.
5. Cepko, C., Ryder, E. F., Austin, C. P., Walsh, C. & Fekete, D. M. (1995) Lineage analysis using retrovirus vectors. *Methods Enzymol.* 254:387–419.
6. Cornetta, K., Moen, R. C., Culver, K., Morgan, R. A., McLachlin, J. R., Sturm, S., Selegue, J., London, W., Blaese, R. M. & Anderson, W. F. (1990) Amphotropic murine leukemia retrovirus is not an acute pathogen for primates. *Hum. Gene Ther.* 1:15–30.
7. Friedrich, G. & Soriano, P. (1991) Promoter traps in embryonic stem cells: A genetic screen to identify and mutate developmental genes in mice. *Genes Dev.* 5:1513–1523.
8. Golden, J. A., Fields-Berry, S. C. & Cepko, C. L. (1995) Construction and characterization of a highly complex retroviral library for lineage analysis. *Proc. Natl. Acad. Sci. USA* 92:5704–5708.
9. Hoxsar, D., Balling, R., Kothary, R., Magli, M. C., Hozumi, N., Rossant, J. & Bernstein, A. (1985) Insertion of a bacterial gene into the mouse germ line using an infectious retrovirus vector. *Proc. Natl. Acad. Sci. USA* 82:8587–8591.
10. Jahner, D., Haase, K., Mulligan, R. & Jaenisch, R. (1985) Insertion of the bacterial gpt gene into the germ line of mice by retroviral infection. *Proc. Natl. Acad. Sci. USA* 82:6927–6931.
11. Kitamura, T., Onishi, M., Kinoshita, S., Shibuya, A., Miyajima, A. & Nolan, G. P. (1995) Efficient screening of retroviral cDNA expression libraries. *Proc. Natl. Acad. Sci. USA* 92:9146–9150.
12. Paulus, W., Baur, I., Boyce, F. M., Breakefield, X. O. & Reeves, S. A. (1996) Self-contained, tetracycline-regulated retroviral vector system for gene delivery to mammalian cells. *J. Virol.* 70:62–67.
13. Rayner, J. R. & Condo, T. J. (1994) A simple and efficient procedure for generating stable expression libraries by cDNA cloning in a retroviral vector. *Mol. Cell. Biol.* 14:880–887.
14. Robertson, E., Bradley, A., Kuehn, M. & Evans, M. (1986) Germ-line transmission of genes introduced into cultured pluripotential cells by retroviral vector. *Nature* 323:445–448.
15. Salter, D. W., Smith, E. J., Hughes, S. H., Wright, S. E. & Crittenden, L. B. (1987) Transgenic chickens: Insertion of retroviral genes into chicken germ line. *Virology* 157:236–240.
16. Wong, B. Y., Chen, H., Chuong, S. W. & Wong, P. M. (1994) High-efficiency identification of genes by functional analysis from a retroviral cDNA expression library. *J. Virol.* 68:5523–5531.
17. Zannettino, A. C., Rayner, J. R., Ashman, L. K., Condo, T. J. & Simmons, P. J. (1996) A powerful new technique for isolating genes encoding cell surface antigens using retroviral expression cloning. *J. Immunol.* 156:611–620.

ATCC

# MATERIAL SAFETY DATA SHEET

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

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

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

**Description**

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

**SECTION I**
**Hazardous Ingredients**

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

**SECTION II**
**Physical data**

Pink or red aqueous liquid

**SECTION III**
**Health hazards**

**For Biosafety Level 1 Cell Lines**

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

**For Biosafety Level 2 Cell Lines**

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

**SECTION IV**
**Fire and explosion**

Not applicable

**SECTION V**
**Reactivity data**

Stable. Hazardous polymerization will not occur.

**SECTION VI**
**Method of disposal**

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.
Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Follow all Federal, State and local regulations.

**SECTION VII**
**Special protection information**

**For Biosafety Level 1 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

**For Biosafety Level 2 Cell Lines**

Handle as a potentially biohazardous material under at least Biosafety Level 2 containment. Cell lines derived from primate lymphoid tissue may fall under the regulations of 29 CFR 1910.1030 Bloodborne Pathogens.

**SECTION VIII**
**Special precautions or comments**

ATCC recommends that appropriate safety procedures be used when handling all cell lines, especially those derived from human or other primate material. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming, et al., 1995) the ATCC manual on quality control (Hay, et al., 1992), the Journal of Tissue Culture Methods (Caputo, 1988), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

**THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.**


February 2002

ATCC® MATERIAL SAFETY DATA SHEET

# MSDS FOR ATCC MICROBIAL CULTURES (Biosafety Level 1)

**ATCC cultures are not hazardous as defined by OSHA 1910.1200. However, as living microorganisms they are potential biohazards.**

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

**Description**

ATCC microbial cultures consist of all bacteria, fungi, plant and animal viruses, and molecular biology materials such as hosts, vectors, clones and libraries.

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

**SECTION I**
**Hazardous Ingredients**

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

**SECTION II**
**Physical data**

Liquid or solid suspensions; frozen liquid suspensions; freeze-dried.

**SECTION III**
**Health hazards**

This culture is not known to cause disease in healthy human adults or animals.

**SECTION IV**
**Fire and explosion**

Not applicable

**SECTION V**
**Reactivity data**

Stable. Hazardous polymerization will not occur.

## SECTION VI
**Method of disposal**

Spill: Contain the spill and decontaminate using suitable disinfectants such as chlorine bleach or 70% ethyl or isopropyl alcohol.

Waste disposal: Dispose of cultures and exposed materials by autoclaving at 121°C for 20 minutes. Dispose of sealed vials of freeze-dried material by dry heat sterilization at 170°C for four hours.

Follow all Federal, State and local regulations.

## SECTION VII
**Special protection information**

**For Biosafety Level 1 Microbial Cultures**

Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

## SECTION VIII
**Special precautions or comments**

ATCC recommends that all ATCC microbial cultures be handled by qualified microbiologists using appropriate safety procedures and precautions. Detailed discussions of laboratory safety procedures are provided in **Laboratory Safety: Principles and Practice** (Fleming et al., ASM Press, Washington, DC, 1995), and in the U.S. Government Publication, **Biosafety in Microbiological and Biomedical Laboratories** (CDC, 1999). This publication is available in its entirety in the Center for Disease Control Office of Health and Safety's web site at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Information on the classification of human etiologic agents on the basis of hazard can be found as Appendix B in the NIH **Guidelines for Research Involving Recombinant DNA Molecules** at http://grants.nih.gov/grants/policy/recombinentdnaguidelines.htm.

**THE ABOVE INFORMATION IS CORRECT TO THE BEST OF OUR KNOWLEDGE. ALL MATERIALS AND MIXTURES MAY PRESENT UNKNOWN HAZARDS AND SHOULD BE USED WITH CAUTION. THE USER SHOULD MAKE INDEPENDENT DECISIONS REGARDING THE COMPLETENESS OF THE INFORMATION BASED ON ALL SOURCES AVAILABLE. ATCC SHALL NOT BE HELD LIABLE FOR ANY DAMAGE RESULTING FROM HANDLING OR CONTACT WITH THE ABOVE PRODUCT.**


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