THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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

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

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

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

PRINCIPAL INVESTIGATOR	Fred Dick
DEPARTMENT	Biochemistry
ADDRESS	A4136 Victoria Research Labs
PHONE NUMBER	58620
EMERGENCY PHONE NUMBER(S)	519-438-7463
EMAIL	fdick@uwo.ca

Location of experimental work to be carried out: Building(s) _LRCP/VRL_____ Room(s)_4th and 7th floors_

*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:__CIHR and CRS_____
GRANT TITLE(S): __1) Regulation of heterochromatin by the Retinoblastoma protein in cell cycle control and cancer ___2) Differential Control of E2F Transcription Factors by pRB in Normal and Cancer Cells____
3) The role of the retinoblastoma protein in TGF-beta induced growth arrest._____

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

Name	UWO E-mail Address	Date of Biosafety Training
Matt Cecchini	mcecchi2@uwo.ca	5/2007
Srikanth Telluri	stellur@uwo.ca	9/2006
Courtney Coschi	ccoschi@uwo.ca	7/2007
Jasmyne Carnevale	jcarnev@uwo.ca	9/2009

Control of Cell Proliferation in Development and Cancer.

My laboratory studies the genes and regulatory networks that control cell division. Cells have the ability to sense their external surroundings and integrate this information to determine when it is appropriate to divide. The genes that participate in this decision making process are often disrupted in cancer and this contributes to the unregulated cell division that is characteristic of this disease.

Our research work focuses on the genes and their encoded proteins that participate in this growth control process. A key component in controlling cell proliferation is the retinoblastoma protein (pRB) that integrates information from many internal and extracellular signals to control proliferation. We use a combination of in vitro biochemical methods, cell culture assays, and gene targeted mice to understand the mechanisms used by the retinoblastoma protein and other growth regulators to control proliferation and relate their function to cancer susceptibility in mice.

In order to undertake this type of research we use many modern molecular biological tools (and generate their associated biohazards). We are frequently manipulating gene sequences using recombinant DNA technology. We also utilize bacteria extensively for recombinant protein expression. These reagents are used in experiments to help us develop models of biochemical function that we test in mammalian cell culture experiments. Experiments in mammalian cells often involve re-expressing wild type or mutant forms of proteins like pRB in cells that are deficient for its function. We can then investigate its function by studying the proteins pRB interacts with in extracts from these cells, or we can assay cellular outcomes such as proliferation or apoptosis. Lastly, the most intriguing mutants from these studies are used to create gene-targeted mouse strains so that we can relate the proliferative effects that we see in cell culture with cell cycle control in development. Most importantly, we can determine if these defects in proliferative control in mice is manifest in cancer.

Recently, we have also begun to collect DNA samples derived from peripheral blood, or primary human tumors. We are using these samples to search for new genetic alterations in growth regulating components like pRB. In this way we are hoping to relate mechanisms of growth control characterized in our research with the genetic characteristics of human cancers to better classify cancer patients for treatment.

Disposal of biohazardous materials

The experimental approach that is described above utilizes, or generates, biohazards that fit into the following categories:

Bacterial cell cultures that harbor foreign DNA:
All solid phase media and culture vessels are sealed in biohazardous waste containers for autoclaving. All liquid cultures are bleached, neutralized, and disposed of down the drain.

Mammalian cell culture (primary and immortal cell isolates):
All tissue culture plastics are sealed in biohazardous waste containers for autoclaving. Liquid waste is bleached, neutralized, and disposed of down the drain.

Viral production (and associated mammalian cell culture):
All tissue culture plastics are collected in biohazardous waste bags inside the laminar flow hood, sealed, and autoclaved after removal from the hood. All glassware is disinfected with bleach inside the laminar flow hood and neutralized before removal from the hood. All liquid waste is bleached, and neutralized inside the hood before removal.

Extraction of DNA from human tissues:
All extractions are carried out in a laminar flow hood. We use an agent called 'Trizol' that dissolves the tissue and allows the DNA to be isolated. Its harsh chemical make up destroys any associated pathogens that may be present. Dissolved tissue is disposed of in biohazardous waste containers for autoclaving. The DNA is further purified and sterilized in alcohol and stored.

1.0 Microorganisms

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

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

_____

_____

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

_____

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E. coli (DH5α,BL21, BS1365, and derivatives of these strains)	☐ Yes X No	☐ Yes X No	☐ Yes X No	8 litres	ATCC/ other investigators	X 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 (*Data sheet for DH5α is provided as an example for all E. coli*).

2.0 Cell Culture

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

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes X No		Not applicable
Rodent	X Yes ☐ No	Embryos and organs from our mice	2007-058
Non-human primate	☐ Yes X No		
Other (specify)	☐ Yes X No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	X Yes ☐ No	C33A, Saos-2, U2OS, HeLa, H1299, T98G, IMR90, Phoenix-Eco	ATCC, other investigators
Rodent	X Yes ☐ No	NIH 3T3, other 3T3's	ATCC, and our primary cultures
Non-human primate	☐ Yes X No		
Other (specify)	☐ Yes X No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) (*Data sheet for C33A is provided as an example for all human cell lines*).

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? X 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/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		O 1 O 2 ☐ 2+ O 3
Human Blood (fraction) or other Body Fluid	LHSC molecular diagnostics lab	☐ Yes X Unknown		O 1 X 2 ☐ 2+ O 3
Human Organs or Tissues (unpreserved)	OICR tumor bank	☐ Yes X Unknown		O 1 X 2 ☐ 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or transfection
DH5a, BL21, BS1365	See accompanying table of plasmids.	See accompanying table of plasmids.	Too numerous to list, in general related to cell division (eg. Ras and E7) and viability	None in bacteria. In mammalian cells we see growth arrest, growth acceleration, or apoptosis.

* Please attach a Material Data Sheet or equivalent if available. (*Data sheet for DH5α is provided as an example*).
** Please attach a plasmid map. (*See accompanying table of plasmids*).

4.3 Will genetic modification(s) involving viral vectors be made? X 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
none	ecotropic retroviruses, recombinant adenovirus	other investigators	as above (including Ras and E7)	In mammalian cells we see growth arrest, growth acceleration, or apoptosis. We also use viruses containing cre to delete gene sequences containing LoxP sites.

* Please attach a Material Safety Data Sheet or equivalent. (*Data sheet for a recombinant adenovirus from a company is provided as an example, none available for retroviruses*)

4.4 Will genetic sequences from the following be involved?

- HIV ☐ YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ○ YES, specify _____ X NO
- SV 40 Large T antigen ☐ YES X NO
- E1A oncogene X YES ☐ NO
- Known oncogenes X YES, please specify __E7, Ras_____ ☐ NO
- Other human or animal pathogen and or their toxins ☐ YES, please specify _____ X NO

4.5 Will virus be replication defective? X YES ☐ NO

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

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

(Please note that oncogenes will only be packaged into ecotropic viruses that will be used to infect murine cells (or human cells engineered to carry the ecotropic receptor). All work will be done in containment level 2. Adenoviruses will not carry oncogenes, only growth suppressing genes, or cre. These viruses will be used to infect mice or murine cell cultures.)

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? ☐ YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used: mouse

6.3 AUS protocol # 2007-058

6.4 Will any of the agents listed in section 4.0 be used in live animals X YES, specify: Adenoviruses expressing cre will be used to delete gene sequences from transgenic mouse lines ☐ NO

6.5 Will the agent(s) be shed by the animal: X YES ☐ NO, please justify:
Some replication defective adenovirus is likely to be shed in the days immediately following administration of the virus. Please see animal protocol 2007-058 for details of viral containment during the housing of these mice.

7.0 Use of Animal species with Zoonotic Hazards

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

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

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

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

8.4 How much of the toxin is handled at one time*?

8.5 How much of the toxin is stored*?

8.6 Will any biological toxins be used in live animals? ☐ YES, Please provide details: ☐ NO

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

9.0 Insects

9.1 Do you use insects? ☐ YES X NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

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

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

_____

_____

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

_____

_____

10.0 Plants

10.1 Do you use plants? ☐ YES X NO If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

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

10.6 Do you do any modifications to the plant? ☐ YES ☐ 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? ☐ YES ☐ NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

_____

_____

11.0 Import Requirements

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

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

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

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

12.0 Training Requirements for Personnel Named on Form

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

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

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

SIGNATURE _____

13.0 Containment Levels

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

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

X YES, permit # if on-campus_____
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE _____ Date: Jan. 14 /2011

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

_____None needed_____

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

____We don't inject any of the agents listed above. The greatest risk for people in my lab is contact with these agents on their skin. The best remedy is thorough washing of the affected areas. None of the listed agents offers a serious health risk from skin exposure. Even adenoviruses don't infect through the epidermis (they need mucosal membranes).

15.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: Mike Ryder
Date: January 14, 2011

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

Special Conditions of Approval:

Product Information Sheet for ATCC® 53868™

ATCC® Catalog No. 53868™

Designation: *Escherichia coli* DH5

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.

Description: This is a recA- *Escherichia coli* host strain for plasmids and cosmids.

Lineage: strain 100 (Hoffman-Berling)→ MM294 (Meselson) → DH1 (Hanahan) → DH5 (Hanahan)

Deposited for Patent purposes on behalf of : Cold Spring Harbo Laboratories, Cold Spring Harbor, NY

Genotype : F- supE44 hsdR (rK- mK+) recA1 gyrA96 endA1 thi-1 relA1 deoR λ-

Shipped: Frozen glycerol stock of *E. coli*

Propagation:
Transfer a loopful to a test tube containing 5 mL LB broth. A loopful of culture can also be streaked on an LB agar plate. Incubate cultures at 37° C. Isolate DNA using standard plasmid preparation procedures.

Conditions: Store frozen vial at -80° C.

BIOSAFETY LEVEL: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC® WARRANTY:

The viability of ATCC® products is warranted for 30 days from the date of shipment. If you feel there is a problem with this product, contact Technical Services by phone at 800-638-6597 or 703-365-2700 or by e-mail at tech@atcc.org. Or you may contact your local distributor.

DISCLAIMERS:

This product is intended for laboratory research purposes only. It is not intended for use in humans.

While ATCC® uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC® makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC® does not warrant that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, and use. ATCC® is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC® is not liable for damages arising from the misidentification or misrepresentation of cultures.

Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org.

Updated (06/10) LMH

ATCC® (American Type Culture Collection)
P.O. Box 1549
Manassas, VA 20108 USA
www.atcc.org

800-638-6597 or 703-365-2700
Fax: 703-365-2750
E-mail: tech@atcc.org
Or contact your local distributor.

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

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

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

October 20th, 2009

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

By Facsimile: (289) 288-0020

SUBJECT: Importation of *Escherichia coli* strains

Dear Ms. Survery / Mr. Decosimo:

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

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

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

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

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

Sincerely,

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

Canada

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045
http://www.vectorbiolabs.com

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

3701 Market Street, Suite 340, Philadelphia, PA 19104 Tel: 877-biolabs Fax: 215-966-6001 info@vectorbiolabs.com

Cell line info

Cell Biology

ATCC® Number:	CRM-HTB-31™ Order this Item	Price:	$400.00

Designations:	C-33 A
Depositors:	N Auersperg
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	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:	For use in testing and calibration in ISO 17025 accredited laboratories, to challenge assay performance, validate or compare test methods, and to establish sensitivity, linearity and specificity during assay validation or implementation. ISO Guide 34:2000<\a.
Tumorigenic:	YES
Oncogene:	p53 +; pRB +
DNA Profile (STR):	Amelogenin: X CSF1PO: 12 D13S317: 13 D16S539: 13,14 D5S818: 11,12 D7S820: 10 THO1: 7,8 TPOX: 9 vWA: 18,20
Cytogenetic Analysis:	This a pseudodiploid human cell line with the modal chromosome number of 46, occurring in 70% of cells examined. Polyploid cells occurred at 8.6%. Seven marker chromosomes were consistently detected per pseudodiploid cell. They are: t(1q17q), t(1p21q), del(18) (q21.3), der(1)t(1;17) (p16;q21.3) and three others. Several other markers were also found but they occurred only once in 15 metaphases analyzed. Neither DMs nor HSRs were detected. Structurally normal N1 was absent. Generally there are two X chromosomes in each cell.
Isoenzymes:	AK-1, 1 ES-D, 1
Age:	66 years adult

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Cell Biology

ATCC® Number:	HTB-85™ Order this Item	Price:	$269.00

Designations:	Saos-2
Depositors:	J Fogh, G Trempe
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** bone **Disease:** osteosarcoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The cells are distributed for research purposes only. The Memorial Sloan-Kettering Cancer Center releases the line subject to the following: 1.) The cells or their products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of these cells must first be negotiated with The Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	epidermal growth factor (EGF); transforming growth factor beta (type 1 and type 2)
Tumorigenic:	No
Antigen Expression:	Blood Type B, Rh+; HLA A2, A3, Bw16, Bw47
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 12,13 D16S539: 12,13 D5S818: 12 D7S820: 8,10 THO1: 6,9 TPOX: 8 vWA: 18

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Cell Biology

ATCC® Number:	**HTB-96™** Order this Item	Price:	**$256.00**

Designations:	U-2 OS
Depositors:	Hellstrom
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** bone **Disease:** osteosarcoma
Cellular Products:	osteosacoma derived growth factor (ODGF)
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	insulin-like growth factor I (IGF-I); insulin-like growth factor II (IGF II)
Antigen Expression:	Blood Type A; Rh+; HLA A2, Aw30, B12, Bw35, B40(+/-)
DNA Profile (STR):	Amelogenin: X CSF1PO: 13 D13S317: 13 D16S539: 11,12 D5S818: 11 D7S820: 11,12 THO1: 6,9.3 TPOX: 11,12 vWA: 14,18
Cytogenetic Analysis:	Cell line U-2 OS is chromosomally highly altered, with chromosome counts in the hypertriploid range. We did not find the hypodiploid cell population described by J. Ponten, et al., Instead, most of the population has slightly higher counts than first described. Very few normal chromosomes are present, but a high number of stable marker chromosomes are identified., Different chromosomal rearrangements involving the same chromosomes (N1, N7, N9, and N11 particularly), are seen. Twenty-two markers are found including: t(9qter---->9q21::1p36--->1p::?), 7p+, iso(17q), t(15q;?), 4q+, del(3)(q21), 5q(aberrant) and others. [22509]

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Cell Biology

ATCC® Number:	**CRL-5803™**	Order this Item	Price:	**$269.00**

Designations:	NCI-H1299
Depositors:	AF Gazdar, JD Minna
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** lung **Disease:** carcinoma; non-small cell lung cancer **Derived from metastatic site:** lymph node
Cellular Products:	neuromedin B
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The line is available with the following restrictions: 1. This cell line was deposited at the ATCCby Dr. A. Gazdar and Dr. J. Minna and is provided for research purposes only. Neither the cell line nor 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 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 these cells, or their products must first be negotiated with the University of Texas Southwestern Medical Center at Dallas, 5323 Harry Hines Blvd., Dallas, Texas 75235. Telephone (214) 699-8056, FAX (214) 688-7233.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
DNA Profile (STR):	Amelogenin:X CSF1PO:12 D13S317:12 D16S539:12,13 D5S818:11 D7S820:10 THO1:6,9.3 TPOX:8 vWA: 16,17,18
Age:	43 years adult

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Cell Biology

ATCC® Number:	**CCL-186™**	Order this Item	Price:	**$256.00**

Designations:	IMR-90
Depositors:	WW Nichols
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast
Source:	**Organ:** lung **Disease:** normal **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza)
Virus Susceptibility:	Human poliovirus 1 Human poliovirus 2 Varicella-Zoster Herpes simplex virus 1 Herpes simplex virus 2 Human poliovirus 3 Vaccinia virus Human herpesvirus 5 Vesicular stomatitis virus
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,13 D13S317: 11,13 D16S539: 10,13 D5S818: 12,13 D7S820: 9,12 THO1: 8,9.3 TPOX: 8,9 vWA: 16,19
Cytogenetic Analysis:	normal human female; diploid; stable
Isoenzymes:	G6PD, B
Age:	16 weeks gestation
Gender:	female
Ethnicity:	Caucasian

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Cell Biology

ATCC® Number:	**CRL-1690™**	Order this Item	Price:	**$264.00**

Designations:	T98G [T98-G]
Depositors:	GH Stein
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	fibroblast
Source:	**Organ:** brain **Disease:** glioblastoma multiforme
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Roche FuGENE® Transfection Reagents)
Tumorigenic:	No
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 13 D16S539: 13 D5S818: 10,12 D7S820: 9,10 THO1: 7,9.3 TPOX: 8 vWA: 17,20
Cytogenetic Analysis:	This is a human cell line with hyperpentaploid chromosome count. The modal chromosome number should be around 128 to 132. The rate of cells with higher ploidies was 1.39%. Fourteen to 16 marker chromosomes were common to most cells. They were: der(1)t(1;?) (p36;?), i(6p), der(10)t(10;?) (q24;?), der (19)t(19;?) (q13;?), der(15)t(15;?) (q26?;?), minute metacentric and eight to ten others. Most of these structurally altered markers had complex interchromosomal exchanges. The der(10) and der(19) could be formed from a balanced translocation, i.e., t(10;19) (q24;q13). These two markers and the minute metacentric were present in three or more copies in most cells. There were six or more copies for N5, N7, N11, N13, N20, N21, and N22 in most cells. The X and N15 had only one copy.
Age:	61 years
Gender:	male
Ethnicity:	Caucasian

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Cell Biology

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

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial 
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18

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Cell Biology

ATCC® Number:	**CRL-1658™**	Order this Item	Price:	**$279.00**

Designations:	NIH/3T3
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast 
Source:	**Organ:** embryo **Strain:** NIH/Swiss **Cell Type:** fibroblast fibroblast;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Murine leukemia virus
Age:	embryo
Comments:	The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457]. Tested and found negative for ectromelia virus (mousepox).
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: bovine calf serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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orbigen

Phoenix™ Eco Cells (Murine)

PRODUCT SUMMARY

Cat. No:	**RVC-10002**
Quantity:	1 vial of 10^6 Phoenix™ Eco Cells
Storage:	Store in liquid nitrogen.
Stability:	See Protocol for proper procedure in cells handling.

DESCRIPTION

Phoenix™ Eco packaging cell lines was created by placing constructs which is capable of producing gag-pol and envelope protein for amphotropic viruses into 293T cells.. This cell line offeres the great advantages over previous stable systems in that virus can be produced in just a few days. Orbigen's Phoenix™ Eco cells have been extensively tested for helper virus production and established as being helper-virus free.

Gag-pol was introduced with hygromycin as the co-selectable marker.The envelope proteins were introduced with diptheria toxin resistance as the co-selectable marker. An IRES-CD8 surface marker was also introduced downstream of the reading frame of the gag-pol construct to monitor gag-pol production which can be readily monitored by flow cytometry.

Eotropic packaging cells system is to deliver genes to dividing cells of murine or rat.

PROTOCOLS:

Thawing Phoenix™ Eco Cells:

1. Remove the vial containing frozen cells from liquid nitrogen or shipping box. Thaw rapidly at 37°C by holding the vial and gently shaking in the water bath. Take out the vial from the water bath when the frozen cells start to thaw (about 1-2 minutes). The key point is NOT to let the cells thaw completely.
2. Immediately add 1 ml of Growth Medium (High glucose DMEM containing 10% heat inactivated fetal bovine serum, 100 U/ml Penicillin, 100 U/ml Streptomycin, 2 mM L-Glutamine) to the cells and gently transfer them to a 15 ml sterile conical screw cap tube.
3. Add 2 ml of GM and gently mix the cells to allow osmotic equilibration.
4. Add 10 ml of GM, close the tube, invert the tube several times and spin cells at 500 x g for five minutes.
5. Remove the supernatant, resuspend cell pellet in GM, and transfer cells to a 10 cm tissue culture dish.

Note: *It is important to freeze multiple vials of each producer cell line after first receiving and expanding them to ensure a ready supply of backup vials to allow for uniform virus production over several years. If the cells are to be carried in selective media, this should not be applied until after the first passage.*

Growth and passage of Phoenix™ Eco cells:

Phoenix™ Eco cells derived from 293 cells are carried in GM and grown in a 37°C incubator supplied with 5% CO_2. To split and passage the cell lines:

1. Gently rinse cultured cells 1x with PBS (without Ca++ or Mg++).
2. Trypsinize (.05% trypsin/0.53 mM EDTA) until the cells easily detach and can be readily pipetted into a single cell suspension.
3. Trypsinization is quenched with GM prior to subculture in fresh medium.

Note: *Do not split the cells at densities more dilute than 1:5 in order to maintain the uniformity of the cells in culture and minimize the outgrowth of clonal variants. The cells should not be allowed to grow over-confluent. This leads to the formation of cell clumps in culture which can cause uneven cell distribution after replating and result in less efficient transfection.*

Passaging Phoenix™ Eco cells:

To achieve optimal cell conditions, passage cells at 1:4 or 1:5 at 70-80% confluent every 2-3 days. Never let cells reach confluence since this will reduce transfection efficiency in the short term. Passage of Phoenix™ Eco cells every few months in Hygromycin (300 µg/ml) and Diptheria Toxin (1µg/ml) containing medium for one week is recommended.

Cells can be analyzed and sorted by fluorescent activated cell scan (FACS) for expression of mouse CD8 (a proxy measure of gag-pol in this cell line) and for surface expression of envelope protein with 83A25 antibody.

*****Special Note:***

This product is only available for non-profit organization researches. Industrial customers will need to obtain a license agreement with Stanford University prior ordering this product from Orbigen.