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

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

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

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

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

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

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

PRINCIPAL INVESTIGATOR:	**Dr. Hon Sing Leong**
DEPARTMENT:	**CRL LRCP**
ADDRESS:	**800 Commissioners Rd East**
PHONE NUMBER:	**519-686-8500 Ex.56474**
EMERGENCY PHONE NUMBER(S):	
EMAIL:	**honsing.leong@gmail.com**

Location of experimental work to be carried out :

Building :	**London Regional Cancer Program**	Room(s):	**A4-829, A4-823, A4-909**
Building :		Room(s):	
Building :		Room(s):	

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

FUNDING AGENCY/AGENCIES: **Prostate Cancer Canada, Ontario Cancer Research Institute**

GRANT TITLE(S): **Non-invasive staging of prostate cancer: detection of circulating prostate microparticles using unique metastasis-specific antibody 1A5; Translating the Clinical Utility of Cancer Microparticle and Circulating Tumour Cell Enumeration and Functional**

UNDERGRADUATE COURSE NAME(IF APPLICABLE): **Tumor Imaging for Prognosis of CRC.**

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

Name	UWO E-mail Address	Date of Biosafety Training
Colleen Biggs	**cbiggs4@uwo.ca**	**May 2012**
Siddika Pardhan	**spardha@alumni.uwo.ca**	**May 2012**
Connor MacMillan	**cmacmil8@gmail.com**	**October 2010**

Sean Leith	sleith@uwo.ca	May 2012
Jenny Ma	jenny.ma@lhsc.on.ca	June 2011
Allen Clifford	acliffo4@uwo.ca	October 2011

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

Biological agents such as antibodies and peptides are non-biohazardous and are typically disposed of by pre-treatment with bleach and then put down the sink. These biological agents are always stored in locked tubes in either a -20C freezer or +4C fridge. These are fridges that are designed for storage of biological agents only.

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

The goal of our laboratory group is to define mechanisms of bone metastasis in prostate cancer patients, to develop novel imaging agents that target micrometastatic lesions and to develop and bring into clinical use prognostic and diagnostic tests centered on the enumeration of cancer and bone microparticles which are released at key steps of metastasis. I have established techniques for the intravital imaging of key steps of metastasis and the enumeration of plasma microparticle of various cell origins. We perform techniques such as flow cytometry, immunohistochemistry of cells and tissue sections and western immunoblotting.

Some of the assays will involve the use of patient plasma and other bodily fluids such as urine. These fluids will be handled using level II biohazard handling techniques and will thoroughly bleached before disposing. This has been suggested by the Ethics Review Committee and patient consent will always be obtained. All patient plasmas are anonymized and all clinical data only known by a study coordinator. For flow cytometry, all antibodies used are obtained commercially. Tissue sections will be acquired by hospital pathology departments and stained according to their immunohistochemistry protocols. Bacteria is used for the production and isolation of vector DNA for transfection into mammalian cancer cell lines for testing scientific hypotheses. All plasmid maps have been attached to this application. We do not use viruses for our research and do not intend to in the near future. No primary tissues or fluids from patients will be used for culture in animal models. For all animal models, we do not use chemotherapy drugs or any cytotoxic agents for our research. All animals and their tissues and bodily fluids will be disposed of according to animal protocols. No animal husbandry is necessary as we will purchase mice for each experiment. We do not intend to start mouse colonies for our experiments.

1.0 Microorganisms

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

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

Please describe the risk (if any) of escape and how this will be mitigated:
In the event of any leakage or spillage of fluids used to grow bacteria for molecular biology purposes, 20% bleach and then 75% ethanol will be used to clean up the affected area.

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Escherichia Coli	☒ Yes ☐ No	☒ Yes ☐ No	☒ Yes ☐ No	**0.05L***	**Invitrogen**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☒ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

DH5-alpha

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

Additional Comments: *** approximately 1 x 10 9 cells in total in 50 mL volume. MSDS is on above link.**

2.0 Cell Culture

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

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

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**HEp3B, LNCap HT1080, PC3, RT4, T24**	**Hep3B level 2. Rest are level 1**	**ATCC (all)**
Rodent	☐ Yes ☒ No			
Non-human primate	☐ Yes ☒ No			
Other (specify)	☐ Yes ☒ No			

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

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

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

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

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
DH5alpha	**TrueClone vectors encoding STEAP, UP1A, UPII**	**OpenBiosystems Inc.**	**STEAP, UP1A, UPII**	**no**	**no**	**none, just production of vector**

E. coli

equivalent if available.

r the following strains of E. coli:
docs/ohs/CFIA_Ecoli_list.pdf

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

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

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

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

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

5.0 Will genetic sequences from the following be involved?

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

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

Additional Comments: _____

6.0 Human Gene Therapy Trials

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

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

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

6.4 How will the biological agent be administered?

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

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

7.0 Animal Experiments

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

7.2 Name of animal species to be used **Mus musculus**

7.3 AUS protocol # **not yet applied**

7.4 List the location(s) for the animal experimentation and housing. **7th Floor animal facility, VRL, LRCP**

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

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

8.0 Use of Animal species with Zoonotic Hazards

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

8.2 Will live animals be used? ☐ YES ☒ NO

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

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

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

9.0 Biological Toxins and Hormones

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

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

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

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

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

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

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

Additional Comments: _____

10.0 Insects

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

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

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

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

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

11.0 Plants

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

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

11.3 What is the origin of the plant?

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

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

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

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

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

12.0 Import Requirements

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

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

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

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

13.0 Training Requirements for Personnel Named on Form

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

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

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name HSC ***Date:*** 9/7/12

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **December 10, 2012**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants): **R-06-000599**

15.0 Procedures to be Followed

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

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Staff have been trained to do the following: get immediate medical attention at either LHSC Occupational Health and Safety or Victoria Emergency, visit Occ Health as soon as possible, and file an LHSC incident report. UWO employees are asked to visit UWO Occ Health to file an incident report.

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

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name Hon Leung ***Date:*** 9/7/12

15.4 Additional Comments: _____

16.0 Approvals

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

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

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: Sept 14, 2012

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

Special Conditions of Approval:

Product Information Sheet for ATCC® HB-8064™

Cell Line Designation: Hep 3B 2.1-7
ATCC® Catalog No. HB-8064™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: liver; hepatocellular carcinoma
Age: 8 years
Gender: male
Ethnicity: black
Morphology: epithelial
Growth properties: adherent
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 8
D13S317: 12, 14
D16S539: 10
D5S818: 13
D7S820: 8, 10
TH01: 6, 7
TPOX: 9
vWA: 17
Tumorigenic: yes, forms tumors in nude mice
Products: alpha fetoprotein (alpha-fetoprotein); hepatitis B surface antigen (HBsAg); albumin; alpha2 macroglobulin (alpha-2-macroglobulin); alpha1 antitrypsin (alpha-1-antitrypsin); transferrin; alpha1 antichymotrypsin (alpha-1-antichymotrypsin); haptoglobin; ceruloplasmin; plasminogen; complement (C3, C4); C3 activator; fibrinogen; alpha1 acid glycoprotein (alpha-1 acid glycoprotein); alpha2 HS glycoprotein (alpha-2-HS-glycoprotein); beta lipoprotein (beta-lipoprotein); retinol binding protein (retinol-binding protein); Gc globulin
Depositors: The Wistar Institute
Comments: This line contains an integrated hepatitis B virus genome. ATCC confirmed this cell line tested positive for the presence of HepB viral DNA sequences via PCR.
Karyotype: modal number = 60 with a subtetraploid mode of 82; has a rearranged chromosome 1
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Purified DNA from this line is available as ATCC HB-8064D™ (10µg)

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories,* 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These cells are distributed for research purposes only. Cell lines and hybridomas deposited for patent purposes are not always screened for contamination, antibody production or characterized by the ATCC. Release of a culture, the use of which may be claimed in a patent, from the ATCC during the effective term of any such patent is not meant to carry with it, and does not grant any license, express or implied, under any patent, or the right to use a culture in any process described in a patent.
The above culture was deposited in the ATCC in connection with a patent application. Copies of U.S. Patents may be obtained from the Commissioner of Patents, U.S. Patent and Trademark Office, Box 9, Washington, D.C. 20231. This cell is the subject of U.S. Patent 4,393,133.
This material is cited in a U.S and/or other Patent and may not be used to infringe the patent claims.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or

Product Information Sheet for ATCC® CRL-1740™

Cell Line Designation:
LNCaP Clone FGC
ATCC Catalog No. CRL-1740™

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: prostate carcinoma; metastatic site: left supraclavicular lymph node
Age: 50 years
Gender: male
Ethnicity: caucasian
Morphology: epithelial
Growth Properties: clusters; lightly adherent
Doubling time: about 34 hours
Tumorigenic: yes, in nude mice
Products: human prostatic acid phosphatase; prostate specific antigen (PSA)
Receptors expressed: androgen; estrogen
DNA profile (STR analysis):

Amelogenin: X,Y
CSF1PO: 10,11
D13S317: 10,12
D16S539: 11
D5S818: 11,12
D7S820: 9.1,10.3
TH01: 9
TPOX: 8,9
vWA: 16,18

Depositors: J.S. Horoszewicz
Comments: This line was isolated in 1977 by Horoszewicz et al from a needle aspiration biopsy of the left supraclavicular lymph node of a 50-year-old Caucasian male with confirmed diagnosis of metastatic prostate carcinoma. These cells are responsive to 5-alpha-dihydrotestosterone (growth modulation and acid phosphatase production).

The cells may not produce a uniform monolayer, but grow in clusters which should be broken apart by repeated pipetting when subcultures are prepared. They attach only lightly to the substrate, may not become confluent and rapidly acidify the medium. Growth is very slow. The cells should be allowed to incubate undisturbed for the first 48 hours after subculture. When flask cultures are shipped, the majority of the cells become detached from the flask and float in the medium. Upon receipt, incubate the flask (in the usual position for monolayer cultures) for 24 to 48 hours to allow the cells to re-attach. The medium can then be removed and replaced with fresh medium. If desired, the contents of the flask can be collected, centrifuged at 125 xg for 10 minutes, resuspended in 10 ml of medium and dispensed into a single flask.

Karyotype: This is a hypotetraploid human cell line. The modal chromosome number was 84, occurring in 22% of cells. However, cells with chromosome counts of 86 (20%) and 87 (18%) also occurred at high frequencies. The rate of cells with higher ploidies was 6.0%.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA from this line is available as ATCC CRL-1740D™ (10µg)

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: *Biosafety in Microbiological and Biomedical Laboratories*, 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-

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.

ATCC

Product Information Sheet for ATCC® CCL-121™

Cell Line Designation: HT-1080
ATCC® Catalog No. CCL-121

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: fibrosarcoma
Age: 35 years
Gender: male
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
VirusSuscept: poliovirus 1; vesicular stomatitis (Indiana); RD114; feline leukemia virus (FeLV)
Tumorigenic: yes, in immunosuppressed mice
Oncogene: ras +
Reverse Transcriptase: negative
DNA profile (STR analysis)
Amelogenin: X,Y
CSF1PO: 12
D13S317: 12,14
D16S539: 9,12
D5S818: 11,13
D7S820: 9,10
TH01: 6
TPOX: 8
vWA: 14,19.
Isoenzymes: G6PD, B
Depositors: S. Rasheed
Karyotype: Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. 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. 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*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Use Restrictions

These cells are distributed for research purposes only. ATCC recommends that individuals contemplating commercial use of any cell line first contact the originating investigator to negotiate an agreement. Third party distribution of this cell line is discouraged, since this practice has resulted in the unintentional spreading of cell lines contaminated with inappropriate animal cells or microbes.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium. and spin at approximately 125 xg for 5 to7 minutes.

4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be*

Cell Line Designation: PC-3
ATCC® Catalog No. CRL-1435

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: adenocarcinoma; prostate; metastatic site: bone
Age: 62 years
Tumor Stage: grade IV
Gender: male
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
Tumorigenic: yes, Tumors developed within 21 days at 100% frequency (5/5) in nude mice inoculated subcutaneously with 10(7) cells
AntigenExp: HLA A1, A9
Growth Properties: monolayer; the cells form clusters in soft agar and can be adapted to suspension growth
DNA profile (STR analysis):

Amelogenin: X
CSF1PO: 11
D13S317: 11
D16S539: 11
D5S818: 13
D7S820: 8,11
TH01: 6,7
TPOX: 8,9
vWA: 17

Depositors: M.E. Kaighn
Comments: The PC-3 was initiated from a bone metastasis of a grade IV prostatic adenocarcinoma from a 62-year-old male Caucasian. The cells exhibit low acid phosphatase and testosterone-5-alpha reductase activities.
Karyotype: It is near-triploid with a modal number of 62 chromosomes. There are nearly 20 marker chromosomes commonly found in each cell; and normal N2, N3, N4, N5, N12, and N15 are not found. No normal Y chromosomes could be detected by Q-band analysis.
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.
Purified DNA from this line is available as ATCC CRL-1435D (10µg)

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. 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. 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*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

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To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. *It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.*

1. Thaw the vial by gentle agitation in a **37°C** water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a 75 cm^2 tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution

Cell Line Designation: RT-4
ATCC Catalog No. HTB-2

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: urinary bladder; transitional cell papilloma
Age: 63 years
Gender: male
Ethnicity: Caucasian
Morphology: epithelial
Growth properties: adherent
Antigen Expression: HLA A25(10), A3, B12, Cw3; Blood Type O
Tumorigenic: in cheek pouch of steroid-treated hamsters
DNA profile (STR analysis):

Amelogenin: X,Y
CSF1PO: 10,12
D13S317: 8
D16S539: 9
D5S818: 11,12
D7S820: 9,12
TH01: 9,9.3
TPOX: 8,11
vWA: 14,17

Depositors: L. M. Franks; C. Rigby

Biosafety Level: 1

This cell line is not known to harbor an agent known to cause disease in healthy adult humans. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment. 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. 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*** , 4th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 1999. The entire text is available online at www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm

Use Restrictions

These 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.

Handling Procedure for Frozen Cells

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at –70°C. Storage at –70°C will result in loss of viability.

SAFETY PRECAUTION: ATCC highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. ***It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.***

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

3. Transfer the vial contents to a 75 cm^2 tissue culture flask and dilute with the recommended complete culture medium (see the specific batch information for the recommended dilution ratio). *It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).*

4. Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

If it is desired that the cryoprotective agent be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the cell suspension at approximately 125 xg for 5 to 10 minutes. Discard the supernatant and resuspend the cells with fresh growth medium at the dilution ratio recommended in the specific batch information.

ATCC

Product Information Sheet for ATCC® HTB-4™

Cell Line Designation: T24
ATCC® Catalog No. HTB-4

Table of Contents:

Cell Line Description

Organism: *Homo sapiens* (human)
Tissue: bladder; transitional cell carcinoma
Age: 81 years
Gender: female
Ethnicity: Caucasian
DNA profile (STR analysis):
Amelogenin: X
CSF1PO: 10,12
D13S317: 12
D16S539: 9
D5S818: 10,12
D7S820: 10,11
TH01: 6
TPOX: 8,11
vWA: 17
Morphology: epithelial
Growth properties: adherent
Isoenzymes: Me-2, 1-2; PGM3, 1; PGM1, 1; ES-D, 1; AK-1, 1; GLO-1, 1; G6PD, B; Phenotype Frequency Product: 0.0216
Products: tumor specific antigen
Antigen Expression: HLA A1, A3, B18, Bw35, Cw4, DRw2, Dw4
Tumorigenic: yes, in hamster cheek pouch; not in nude mice
Depositors: C. O'Toole
Comments: Leukocytes and sera from patients with transitional cell carcinoma were cytotoxic to T24 and related lines.
Cells have a 19 hour generation time. Contains the ras (H-ras) oncogene.
Karyotype: hypodiploidy to hypopentaploidy; stemline 86; 2 to 4 telocentrics; 3 to 4 minutes, hypotetraploid to hypertetraploid with abnormalities including dicentrics, breaks, pulverization, minutes and telocentric markers
Note: Cytogenetic information is based on initial seed stock at ATCC. Cytogenetic instability has been reported in the literature for some cell lines.

Biosafety Level: 1

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2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. *All of the operations from this point on should be carried out under strict aseptic conditions.*

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.

Notice to purchaser

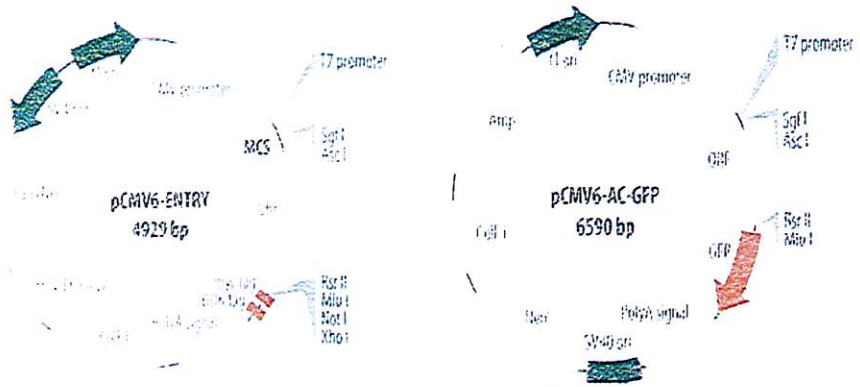
This product is for research use only. Use in and/or for diagnostics and therapeutics is strictly prohibited. By opening and using the product, the purchaser agrees to the following: The plasmids may not be distributed, resold, modified for resale or used to manufacture commercial products without prior written approval from OriGene Technologies, Inc. If you do not agree to the above conditions, please return the UNOPENED product to OriGene Technologies, Inc. within ten (10) days of receipt for a full refund.

Vector Information

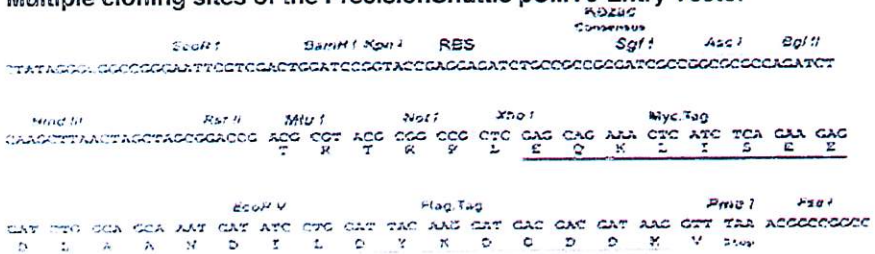
RC123456: TrueORFs with a C-terminal fusion of MYC/DDK tag. The cloning expression vector is pCMV6-Entry. The antibiotic selection marker in E. coli is Kanamycin at 25 ug/ml. Unlike other shuttling systems, the entry vector is a functional mammalian expression vector.

All TrueORF clone vectors are described in detail on OriGene's website at http://www.origene.com/cdna/trueorf/destinationvector.mspx

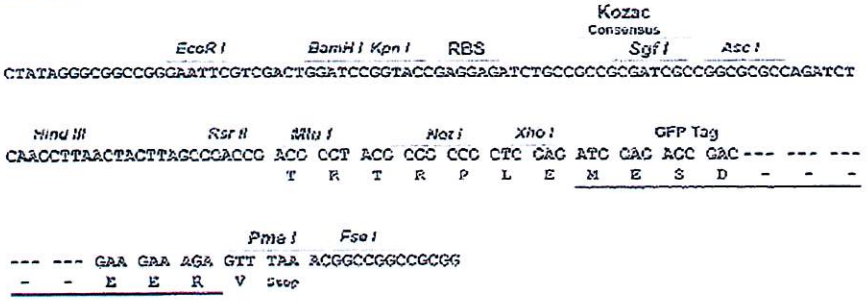
Figure 1. The Vector Maps of pCMV6-Entry and pCMV6-AC-GFP.



Multiple cloning sites of the PrecisionShuttle pCMV6-Entry Vector



Multiple cloning sites in the PrecisionShuttle pCMV6-AC-GFP Vector



The PrecisionShuttle Vector System

The TrueORF vector system is designed to express the ORF of a gene with one or more epitope tags or with a fluorescent marker. There are more than twenty destination vectors in the PrecisionShuttle vector system. These vectors are either N-terminally tagged (pCMV6-AN) or C-terminally tagged (pCMV6-AC). Available tags include Myc, DDK, His, HA, and t-GFP. One untagged PrecisionShuttle vector is also available to express the native (untagged) protein in mammalian cells, or using an *in vitro* protein expression system. Due to the requirement for the Mlu I restriction site, the untagged vector does still append two amino acids (TR) to the C-terminus of the encoded protein.

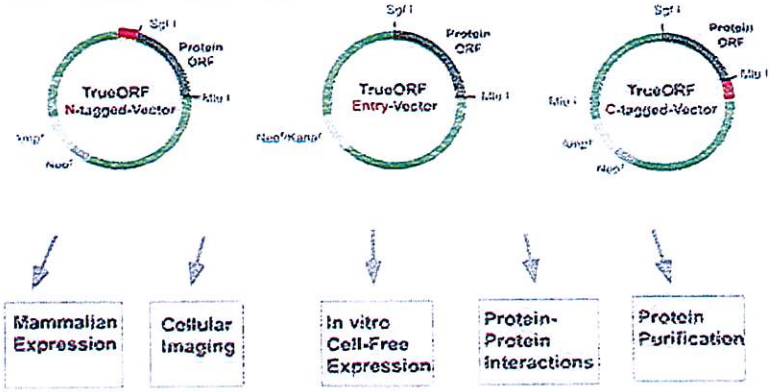


Figure 2. Potential applications of the PrecisionShuttle system for protein analysis. The TrueORF cDNA clones in the pCMV6-Entry vector and other N- or C-tagged-vectors can be directly used in your experiments of protein functional analysis.