Modification Form for Permit BIO-UWO-0227

Permit Holder: Peter Rogan

Approved Personnel
(Please stroke out any personnel to be removed)

Eliseos John Mucaki

Additional Personnel
(Please list additional personnel here)

Heather Tarnowski

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E.coli	
Approved Primary and Established Cells	Human (primary): lymphocyte, fibroblast, hepatocyte. Human (established): lymphoblastoid, HepG2, CaCo2. Rodent (established): rarely. Non-Human primate (established): cos7, cos293, CVI	
Approved Use of Human Source Material	Human blood (whole). Human organs or tissues (unpreserved): small fibroblast sample. Human organs or tissues (preserved): fixed lymphocytes tissue sections.	
Approved Genetic Modifications (Plasmids/Vectors)	PCR 2.1 Topo, pFxP2-DEJT, pDEJT26	pHuR195
Approved Use of Animals		
Approved Biological Toxin(s)		

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. 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 of Permit Holder: _____

Current Classification: 2 **Containment Level for Added Biohazards:** 2

Date of Last Biohazardous Agents Registry Form: Dec 9, 2008

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____ **Date:** _____

ATCC

ATCC Advanced Catalog Search » **Product Details**

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.

Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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ATCC® Number: 61080 Order this Item

Price: $190.00

Designation:	pHuR195 [pHuR 195]
Depositors:	RK Moyzis
Other Id's:	GenBank:M20263
Insert Source:	*Homo sapiens*
Insert Information:	DNA: genomic Insert lengths(kb): 1.200000047683716 Tissue: placenta Gene product: DNA Segment, repetitive [D16Z3]
Biosafety Level:	1
Shipped:	frozen
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.
Vector:	Size (kb): 4.363 Vector: pBR322 (plasmid) Type: cloning Construction: Marker(s):ampR,tetR Construct size (kb): 4.363 Features: marker(s): ampR marker(s): tetR replicon: pMB1
Comments:	Restriction digests of the clone give the following sizes (kb): EcoRI--5.5; HindIII--5.5; BamHI--5.5; PstI--4.3, 1.2; AvaI--5.5. DNA containing this sequence is underrepresented in most genomic libraries because most restriction enzymes (except HinfI and TaqI) generate fragments with pHuR195 elements larger than 20 kb. [5913] This repeat is a variant of the satellite 2 family with a core sequence of CATCAT followed by 4 divergent GGAAT elements with a spacing of 0 - 49 nt. [5913]
Media Description:	ATCC medium 1273: LB medium (ATCC medium 1065) with 20 mcg/ml tetracycline
References:	5913: Moyzis RK, et al. Human chromosome-specific repetitive DNA sequences: novel markers for genetic analysis. Chromosoma 95: 375-386, 1987. PubMed: 3677921

Related Links

- NCBI Entrez Search
- Make a Deposit
- Frequently Asked Questions
- Material Transfer Agreement
- Technical Support
- Media and Antibiotics

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All prices are listed in U.S. dollars and are subject to change without notice. A discount off the current list price will be applied to most cultures for nonprofit institutions in the United States. Cultures that are ordered as test tubes or flasks will carry an additional laboratory fee. Fees for permits, shipping, and handling may apply.

Back to my Search

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Permit Holder: Peter Rogan

Additional Biohazard – Genetic Modification (Plasmid/Vector)

Plasmid pHuR195, purchased from ATCC (Cedarlane Laboratories). See information sheet from ATCC.

This plasmid contains a 1.2 kilobase insert containing satellite repeat DNA from human chromosome 16q11.2. We will be extracting this plasmid from *Escherichia coli,* and using it in fluorescent *in situ* hybridization experiments to identify chromosome 16 in metaphase cells.

Escherichia coli cells containing pHuR195 will be stored as frozen stocks at -80°C. Waste created from cell growth and DNA extraction protocols will passed onto the hazardous waste disposal team on Thursday mornings.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 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. 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 Peter K Rogan, PhD
SIGNATURE Peter K Rogan
DEPARTMENT Biochemistry
ADDRESS MSB 389
PHONE NUMBER x 84255
EMAIL progan@uwo.ca

Location of experimental work to be carried out: Building(s) MSB Room(s) 381, 365A

*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: Start-up funds, Ontario Genomics Institute
GRANT TITLE(S): "Single copy technology for diagnostic + therapeutic applications"

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:

John Macaki
graduate student pending

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 ○ NO
If no, please proceed to Section 2.0

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
E. coli (for recomb. DNA)	O Yes ◉ No	O Yes ◉ No	O Yes ◉ No	< 1 L	*TBD	◉ 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O 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	lymphocyte, fibroblast hepatocyte	Not applicable
Rodent	O Yes ◉ No		
Non-human primate	◉ Yes ◉ No	[illegible]	
Other (specify)	O Yes ◉ No		

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	lymphoblastoid, HepG2, Caco2	ATCC, NIGMS Emp. Coll. Cell established by PI at Rem
Rodent	◉ Yes O No		
Non-human primate	◉ Yes O No	Cos7, 293, CV1	" "
Other (specify)	O Yes ◉ No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) * see attached examples from ATCC + NIGMS

Cultures State + Univ. Missouri Med Schools

2.4 For above named cell types(s) indicate HC or CFIA containment level required O 1 ◉ 2 O 3

3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	study participant (<20 cc.)	○ Yes ● No		○ 1 ● 2 ○ 3
Human Blood (fraction) or other Body Fluid	—	○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (unpreserved)	small fibroblast sample - study participant	○ Yes ● No		○ 1 ● 2 ○ 3
Human Organs or Tissues (preserved)	Fixed lymphocytes tissue sections	○ Yes ● No		● 1 ○ 2 ○ 3

preserved prior to coming to lab by London Health Sci. Centre, Ontario Tumor Bank, collaborating labs

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
competent E. coli cells	PCR 2.1 Topo pENTR-DEST pDEST26	eg Invitrogen	Human cDNA + genomic	copy amplification/ protein expression

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? ○ YES ○ NO Not applicable

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted using the viral vector in 4.0? 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? O YES ◉ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

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 Import Requirements

9.1 Will the agent be imported? O YES, please give country of origin_____ ◉ NO
If no, please proceed to Section 10.0

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

9.3 Has an import permit been obtained from CFIA for animal pathogens? O YES O NO

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

Level 2

10.0 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
- 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 _____

- shared tissue culture with Knoll lab 365A

11.0 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. ● 1 ● 2 ○ 3

live cells

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

○ YES, permit # if on-campus_____
○ NO
○ NOT REQUIRED

fixed preserved cells

MSB 365A - cell culture (D. Litchfield Biochem)

MSB 377 - Not yet certified - To be done

12.0 Procedures to be Followed

12.1 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. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: 7-7-08

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: 9 Dec 2008

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: Dec 4/08

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

Approval Number: B10-UWO-0227 Expiry Date (3 years from Approval): Dec. 09, 2011

Special Conditions of Approval:

My laboratory develops and applies computational methods to interpret single nucleotide polymorphisms and to detect differences in the number of copies of sequences among different individuals. The objectives of my research are to: (a) create computer programs to predict mutations that predispose to disease and then evaluate them by gene and protein expression analysis, (b) to identify and validate genes whose expression responds to drug treatment, and (c) to design and tailor DNA probes to diagnose changes in copy number associated with specific genetic diseases and cancer.

This work utilizes human (and to a lesser extent non-human primate) primary and immortalized cell lines, peripheral blood and fibroblast samples, and suspensions and tissue sections that have been preserved prior to coming to the laboratory. Recombinant human genomic DNA and cDNA are routinely used in the laboratory, some of which are prepared in our own laboratory from nucleic acids extracted from the sources identified above. This entails introduction of plasmids, phage, cosmids and or bacterial artificial chromosomes containing human sequences into laboratory E.coli strains or transfection of common cell lines. Frequently, strains and cell lines containing these cloned constructs are obtained from suppliers such as The Center for Applied Genomics, NIGMS mutant cell repository, American Type Culture Collection or the European Collection of Cell Cultures. If the specific recombinant clone is not available, it will be introduced in to the appropriate vector depending on the length of the target.

The cell lines, peripheral blood and fibroblast samples are used for cell preparation and culture and nucleic acid or protein preparation. Stocks of cell lines and strains are stored in liquid nitrogen cell tanks or ultra low temperature freezers. They will be cultured or handled in a tissue culture facility (MSB 365A, managed by Dr. David Litchfield) prior to fixation and or extraction in the main laboratory (Rm 381, MSB). Lymphoblastod cell lines were developed previously by the PI at other institutions (Penn State College of Medicine and University of Missouri School of Medicine) and through NIGMS or ATCC human genetic cell repository and occasionally, by collaborating researchers.

Peter K. Rogan Ph.D. 2008-07-07