

*Modification Form for Permit BIO-UWO-0002*

*Permit Holder: Gerald Kidder*

**Approved Personnel**

**(Please stroke out any personnel to be removed)**

~~Hong-Xing Wang~~

Poonam Sekhon

Francis Leblanc

Deanne Colley

~~Dan Tong~~

Tony Li

Kevin Barr

**Additional Personnel**

**(Please list additional personnel here)**

**Approved  
Microorganisms**

**Please stroke out any approved  
Biohazards to be removed below**

E. coli (cloning strains)

**Write additional Biohazards for  
approval below. \***

**Approved Cells**

Human (primary), Rodent (primary), ovarian  
follicles, Human (established) HeLa, Rodent  
(established), NRK, N2A

*TH3 mouse Leydig  
cell line (CLI)*

**Approved Use of  
Human Source  
Material**

Blood (whole), unpreserved tissues, ovarian  
follicle cells

**Approved GMO**

Adenovirus vectors, AP2 retroviral vector

\* 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 USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 28, 2008

Signature of Permit Holder



BioSafety Officer(s):

Chair, Biohazards Subcommittee:

*Modification Form for Permit BIO-UWO-0002*

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Approved use of  
Animals

mice

Approved Toxin(s)

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## 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, 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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## Cell Biology

<b>ATCC® Number:</b>	<b>CRL-1714™</b>	<input type="button" value="Order this Item"/>	<b>Price:</b>	<b>\$323.00</b>
<b>Designations:</b>	TM3		<b>Depositors:</b>	JP Mather
<b><u>Biosafety Level:</u></b>	1		<b>Shipped:</b>	frozen
<b>Medium &amp; Serum:</b>	<a href="#">See Propagation</a>		<b>Growth Properties:</b>	adherent
<b>Organism:</b>	<i>Mus musculus</i> (mouse)		<b>Morphology:</b>	epithelial
<b>Source:</b>	<b>Organ:</b> testis <b>Disease:</b> normal <b>Cell Type:</b> Leydig cell;			
<b>Cellular Products:</b>	prostaglandin F2a			
<b>Permits/Forms:</b>	In addition to the <a href="#">MTA</a> mentioned above, other <a href="#">ATCC and/or regulatory permits</a> may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please <a href="#">click here</a> for information regarding the specific requirements for shipment to your location.			
			<b><u>Related Cell Culture Products</u></b>	
<b>Receptors:</b>	luteinizing hormone (LH); epidermal growth factor (EGF); androgen receptor, positive; estrogen receptor, positive; progesterone receptor, positive			
<b>Tumorigenic:</b>	No			
<b>Age:</b>	11 to 13 days			
<b>Gender:</b>	male			
<b>Comments:</b>	The TM3 cell line responds to LH with an increase in cAMP production, but does not respond to follicle stimulating hormone (FSH). The maintenance of responsiveness to LH is dependent upon serum lot. In the presence of LH, the cells are capable of metabolizing cholesterol. Tested and found negative for ectromelia virus (mousepox).			
<b>Propagation:</b>	<b>ATCC complete growth medium:</b> A 1:1 mixture of Ham's F12 medium and Dulbecco's modified Eagle's medium with 2.5mM L-Glutamine, 0.5mM Sodium Pyruvate, 1.2 g/L Sodium Bicarbonate and 15 mM HEPES, 92.5%; horse serum, 5%; fetal bovine serum, 2.5%			
<b>Subculturing:</b>	<b>Protocol:</b> Remove medium, add fresh 0.25% trypsin-0.53mM EDTA solution, rinse, remove trypsin and let the culture sit at room temperature for 2 to 4 minutes. Add fresh medium, aspirate and dispense into new flasks. <b>Subcultivation Ratio:</b> A subcultivation ratio of 1:20 to 1:200 is recommended <b>Medium Renewal:</b> Every 3 to 4 days			
<b>Preservation:</b>	<b>Freeze medium:</b> Complete growth medium, 95%, DMSO, 5% <b>Storage temperature:</b> liquid nitrogen vapor phase			
<b>References:</b>	1158: Mather JP. Establishment and characterization of two distinct mouse testicular epithelial cell lines. Biol. Reprod. 23: 243-252, 1980. PubMed: <a href="#">6774781</a> 1159: Mather JP, et al. Culture of testicular cells in hormone-supplemented serum-free medium. Ann. N.Y. Acad. Sci. 383: 44-68, 1982. PubMed: <a href="#">7046561</a>			

[Return to Top](#)

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**THE UNIVERSITY OF WESTERN ONTARIO  
 BIOHAZARDOUS AGENTS REGISTRY FORM  
 Revised Biohazards Subcommittee: September, 2007**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: [www.uwo.ca/humanresources](http://www.uwo.ca/humanresources)

PRINCIPAL INVESTIGATOR Gerald M. Kidder  
 SIGNATURE G.M. Kidder  
 DEPARTMENT Physiology and Pharmacology  
 ADDRESS 00061B DSB  
 PHONE NUMBER 83132  
 EMAIL gerald.kidder@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 00046 00076 (Child Lab)

\*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Roberts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

GRANT TITLE(S):  
structural determinants of connexin specificity  
Roles of gap junctional coupling in oogenesis  
Non-invasive assessment of oocyte quality  
Cx43 mutations linked to human disease

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

FUNDING AGENCY/AGENCIES NSERC, CIHR, WIF

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

<u>Tony Li</u>	<u>Poonam Sekhon</u>
<u>Kevin Barr</u>	<u>Hong-Xing Wang</u>
<u>Caroline Rahiri</u>	
<u>Dan Tong</u>	
<u>Deanne Colley</u>	
<u>Francois Leblanc</u>	

**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?	Source/ Supplier	Health Canada or CFIA Containment Level
<i>E. coli</i>	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	1 liter	Invitrogen	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
<i>(cloning strains)</i>	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

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

**2.0 Cell Culture**

2.1 Does your work involve the use of cell cultures?  YES  NO

If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>ovarian follicles from IVF clinic</i>
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>ovarian follicles, testes, other organs from mice</i>
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No	
Other (specify)		

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	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>HeLa</i>	<i>colleagues</i>
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	<i>NRK, NZA</i>	<i>"</i>
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

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

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

**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	<i>patient samples</i>	○ Yes ● No		○ 1 ○ 3 ● 2
Human Blood (fraction) or other Body Fluid		○ Yes ○ No		○ 1 ○ 3 ○ 2
Human Organs (unpreserved)		○ Yes ○ No		○ 1 ○ 3 ○ 2
Human Tissues (unpreserved)	<i>Ovarian follicle cells</i>	○ Yes ● No		○ 1 ○ 3 ● 2
Human Organs (preserved)		○ Yes ○ No		○ 1 ○ 3 ○ 2

**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 sequences from the following be involved:  
 ♦ HIV ○ YES ● NO  
 If YES specify \_\_\_\_\_  
 ♦ HTLV 1 or 2 or genes from any CDC class 1 pathogens ○ YES ● NO  
 If YES specify \_\_\_\_\_  
 ♦ Other human or animal pathogen and or their toxins ○ YES ● NO  
 If YES specify \_\_\_\_\_

4.3 Will intact genetic sequences be used from  
 ♦ SV 40 Large T antigen ○ YES ● NO If YES specify \_\_\_\_\_  
 ♦ Known oncogenes ○ YES ● NO If YES specify \_\_\_\_\_

4.4 Will a live viral vector(s) or bacterial plasmid be used for gene transduction ● YES ○ NO  
 If YES name adenovirus vectors  
 Please attach a Material Safety Data Sheet or equivalent.

4.5 List specific vector(s) to be used: AP2 retroviral vector

4.6 Will virus be replication defective ● YES ○ NO

4.7 Will virus be infectious to humans or animals ● YES ○ NO

4.8 Will this be expected to increase the Containment Level required ● YES ○ NO

*level 2 + level 3 procedures: infected cells into mice*

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

**5.0 Human Gene Therapy Trials**

5.1 Will human clinical trials using the viral vector in 4.0 be conducted?  YES  NO

If no, please proceed to Section 6.0

If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus be administered? \_\_\_\_\_

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

5.5 Has human ethics approval been obtained?  YES  NO  PENDING

**6.0 Animal Experiments**

6.1 Will any of the agents listed be used in live animals?  YES  NO

If no, please proceed to section 7.0

6.2 Name of animal species to be used   mice  

6.3 AUS protocol #   2006-061-05  

6.4 If using murine cell lines, have they been tested for murine pathogens?  YES  NO *N/A*

**7.0 Use of Animal species with Zoonotic Hazards**

*primary cells only*

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

- ◆ Pound source dogs  YES  NO
- ◆ Pound source cats  YES  NO
- ◆ Cattle, sheep or goats  YES  NO
- ◆ Non- Human Primates  YES  NO If YES specify species \_\_\_\_\_
- ◆ Wild caught animals  YES  NO If YES specify species \_\_\_\_\_  
colony # \_\_\_\_\_
- ◆ Birds  YES  NO
- ◆ Others (wild or domestic)  YES  NO

**8.0 Biological Toxins**

8.1 Will toxins of biological origin be used?  YES  NO

If no, please proceed to Section 9.0

8.2 If YES, please name the toxin \_\_\_\_\_

8.3 What is the LD<sub>50</sub> (specify species) of the toxin \_\_\_\_\_

8.4 Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

**9.0 Import Requirements**

9.1 Will the agent be imported?  YES  NO  
If no, please proceed to Section 10.0  
If yes, country of origin \_\_\_\_\_

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

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

9.4 Has the import permit been sent to OHS?  YES  NO  
If yes, Permit # \_\_\_\_\_

**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 SM Kildes

**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

11.2 Has the facility been certified by OHS for this level of containment?  YES  NO

11.3 If yes, please give the date and permit number: \_\_\_\_\_

**12.0 Approvals**

UWO Biohazard Subcommittee

Signature Maude Kent Date Mar 28/08

Safety Officer for Institution where experiments will take place

Signature J Stanley, UWO Date March 28/08

Safety Officer for University of Western Ontario (if different from above)

Signature \_\_\_\_\_ Date \_\_\_\_\_

Expiry Date (3 years from Approval): \_\_\_\_\_