

Modification Form for Permit BIO-UWO-0236

Permit Holder: Cheryl Seguin

Approved Personnel

(Please stroke out any personnel to be removed)

Additional Personnel

(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	DH5 alpha competent cells	
Approved Cells	Human (established): CA1/CA2 human embryonic stem cells. Rodent (established): mouse embryonic fibroblasts	CA1/2 genetically modified human embryonic stem cells → express the endoderm transcription factors SOX7 OR SOX17. Endoderm progenitor cell lines.
Approved Use of Human Source Material		
Approved GMO	pClip, pCAGS-ERT2, pCAGS-GRLBD	
Approved use of Animals		
Approved 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 USED.

Classification: 2

Date of last Biohazardous Agents Registry Form: Mar 27, 2009

Signature of Permit Holder: *Cheryl Seguin*

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

The SOX7 and SOX17 expressing HESC lines represent stable lineage-committed progenitors corresponding to the extraembryonic endoderm and definitive endoderm respectively (cells are no longer pluripotent).

They have been shown to demonstrate a stable phenotype over time in culture, and are capable of undergoing subsequent maturation within their respective lineages.

We will be using these cells to understand the molecular pathways responsible for maturation of functional endoderm cell types (such as pancreatic islet cells) through the introduction of specific transcription factor proteins, or culture with selective growth factors.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 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 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 Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

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

Containment Levels will be established 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), (Support Services Building, Room 4190) 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 Cheryle Séguin
SIGNATURE 
DEPARTMENT Physiology and Pharmacology
ADDRESS DSB 0035B
PHONE NUMBER x 82977
EMAIL cheryle.seguin@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 0034, 0023

*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: UWO Startup Funds
GRANT TITLE(S): Directed endoderm differentiation of human embryonic stem cells

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:

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

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:

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

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	Health Canada or CFIA Containment Level
DNA competent cells	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	1L	Invitrogen	<input checked="" 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
	<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	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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	<input checked="" type="radio"/> Yes <input type="radio"/> No	CA1/CA2 human embryonic stem cells	Mount Sinai Hospital, Toronto, ON
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	mouse embryonic fibroblasts	Stem Cell Facility, SickKids Hospital, Toronto
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

2.4 For above named cell types(s) indicate HC or CFIA containment level required 1 2 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		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		<input type="radio"/> Yes <input type="radio"/> No		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

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
DH5α	pCIP pCAG5-ERT2 pCAG5 GFP-LBD	Academic	Pdx-1, NGN3, Nkx2-2, eGFP	endoderm differentiation of human stem cells

* 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 _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

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? 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, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? 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 YES, specify: _____ NO

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? YES NO
If no, please proceed to Section 11.0

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

10.3 What is the origin of the plant? _____

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

10.5 What is your intention? Grow and maintain a crop "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

10.9 Please 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 _____
If no, please proceed to Section 10.0 NO

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 biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE *Khushi Segaria*

13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus _____
 NO
 NOT REQUIRED

done Mar 23/09
gl

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 Charles Seguin Date: March 23/09

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: GM. Kelder
Date: 27 March 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: gl tanley
Date: Mar 27/09

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

Approval Number: BIO-UWO 0236 Expiry Date (3 years from Approval): Mar 27/2012

Special Conditions of Approval:

University of Western Ontario Biohazardous Agents Registry Form

Project Description: Cheryle Séguin, Dept. of Physiology and Pharmacology

Directed differentiation of pancreatic islet precursors from human embryonic stem cells

Diabetes is a chronic disease of insulin insufficiency resulting from autoimmune destruction of pancreatic β cells (Type I), or β cell dysfunction (Type II). While the Edmonton Protocol for islet transplantation is effective, the shortage of donor tissues and the overwhelming number of β cells required for each transplant underscores the need for an alternative source of cells. Human embryonic stem cells (HESC) can potentially generate an unlimited source of insulin-secreting cells for transplantation. However, HESC-based therapies for diabetes require the development of efficient strategies to ensure the specificity of cell differentiation, and the production of fully functional β cells. Although previous studies have used growth factors to promote HESC differentiation, we propose an alternate strategy that exploits the developmental role of regulated transcription factor expression in cell specification. The proposed studies will evaluate the **hypothesis** that pancreatic precursor cells can be generated by the appropriate sequential activation of pancreatic transcription factors in HESC.

Our previous studies have demonstrated that ectopic expression of the endoderm lineage-determining transcription factor SOX17 in HESC is sufficient to produce stable, replicative early endoderm progenitor cells. These studies will use the two established Canadian lines of HESC (CA1 & CA2), developed by Dr. Andras Nagy, Mount Sinai Hospital, Toronto ON (UWO REB number 15646E; SCOC approval attached). We will introduce expression plasmids into HESC to generate stable cell lines with inducible expression of the early pancreatic transcription factors PDX1, NGN3, and NKX2-2, in addition to the endoderm transcription factor SOX17. Following activation of the ectopically expressed transcription factors, cell lines generated in these studies will be evaluated for their phenotype in culture as well as for their ability to produce functional insulin-producing islet cells. The generation of a stable source of β cell precursors and the identification environmental signals required for their maturation may contribute to the generation of the unlimited numbers of insulin-secreting cells required for β cell transplantation.



Office of Research Ethics

The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 5C1
Telephone: (519) 661-3036 Fax: (519) 850-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. C.A. Seguin

Review Number: 15646E

Review Level: Expedited

Review Date: November 04, 2008

Protocol Title: Examining early endoderm specification in pluripotent human stem cells

Department and Institution: Physiology & Pharmacology, UWO

Sponsor:

Ethics Approval Date: November 04, 2008

Expiry Date: December 31, 2019

Documents Reviewed and Approved: UWO Protocol

Documents Received for Information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Humans and the Health Canada/ICH Good Clinical Practice Practices: Consolidated Guidelines; and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced study on the approval date noted above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g. change of monitor, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly also report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse and unexpected experiences or events that are both serious and unexpected;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information, consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB Dr. Joseph Gilbert

Ethics Officer to Contact for Further Information

Janice Sutherland
jsutherland@uwo.ca

Elizabeth Wambolt
ewambolt@uwo.ca

Grace Kelly
grace.kelly@uwo.ca

Denise Grafton
Dgrafton@uwo.ca

This is an official document. Please retain the original in your files.

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November 3, 2008

Dr. Cheryle SEGUIN
Sick Kids Hospital
TMDT Building, rm 13-305
101 College Street
Toronto, Ontario
M5G 1X8

Re: Stem Cell Oversight Committee review of "Examining early endoderm specification in pluripotent human stem cells" (start-up funds from the University of Western Ontario)

Dear Dr. Séguin:

The Stem Cell Oversight Committee (SCOC) met October 7-8, 2008 in Ottawa to review a number of stem cell research applications for conformity to the *Guidelines for Human Pluripotent Stem Cell Research* (<http://www.cihr-irsc.gc.ca/e/34460.html>; "the Guidelines"). SCOC made a number of recommendations that were reviewed and accepted by CIHR's Governing Council in October. The Committee recommended that your request to use the SCOC-approved CA1 and CA2 hESC lines and induced human pluripotent stem cells for this research be approved.

This letter constitutes final approval by CIHR of the above-mentioned project. As CIHR does not notify co-applicants, we ask that you please inform those Individuals and their research institutions (if different from your own) of the outcome of this application.

If in the future you wish to use additional SCOC-approved hESC lines not described in the original SCOC application for this research project, you need only to notify SCOC in writing. You should include the exact title of your original application and date of submission. There will be no need to submit an amended application for SCOC review. If however there are major changes in your research plan, you will need to seek approval by submitting a description of the proposed research involving hES cells. SCOC should also be advised of any conflicts of interest that may arise during the course of this research.

Please remember that you must also have approval from your institution's Research Ethics Board, and your Animal Welfare Committee, before the commencement of your research.

All the best in your research endeavours.

Sincerely yours,

Pierre Chartrand, Ph.D.
Vice-President
Research Portfolio

c.c. John Williams, Chair, Stem Cell Oversight Committee
Kathryn Moore, Director, Governance and Corporate Secretary
Geneviève Dubois-Flynn, A/Director, Ethics Office
Jonathan Faulkner, A/Manager – Program Planning and Analysis

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