

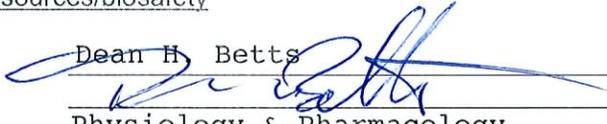
**THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: www.uwo.ca/humanresources/biosafety/

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 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 biohazards being used.

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

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

PRINCIPAL INVESTIGATOR	<u>Dean H. Betts</u>
SIGNATURE	
DEPARTMENT	<u>Physiology & Pharmacology</u>
ADDRESS	<u>Medical Sciences Building M207</u>
PHONE NUMBER	<u>519-661-2111 ext. 83786</u>
EMERGENCY PHONE NUMBER(S)	<u>519-204-3451</u>
EMAIL	<u>dean.betts@schulich.uwo.ca</u>

M298A

Location of experimental work to be carried out: Building(s) MSB, DSB Room(s) DSB 2017A/B
DSB 2025A

*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 12.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC, CHRI, UWO start-up funds

GRANT TITLE(S):
Chromatin remodeling and nuclear reprogramming in domestic animal clones and stem cells
Chemical induction of pluripotent stem cells from human umbilical cord blood

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

<u>Heather Mulholland</u>	_____
<u>Adam Stankiewicz</u>	_____
<u>John Soleas</u>	_____
<u>Lida Radan</u>	_____
<u>Stephanie Hallows</u>	_____

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES NO
 (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:

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

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
VSV-G	<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			0 1 0 2 0 3
retrovirus	<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			0 1 0 2 0 3
lentivirus	<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			0 1 0 2 0 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			0 1 0 2 0 3

} See table 4.3

*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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input checked="" type="radio"/> Yes <input type="radio"/> No	Foreskin	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		

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input checked="" type="radio"/> Yes <input type="radio"/> No	- CA1/CA2/H9 ESCs - Fibroblasts	- Mt Sinai Hospital - Coriell Institute
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	- mouse embryonic fibroblasts	sick kids hospital
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 PHAC 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 Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<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"/> Unknown		<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"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? 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

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

4.3 Will genetic modification(s) 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
VGW-G retrovirus lentivirus	*see attached	data sheet	c-Myc, Klf4 Oct-4, Sox2	induced pluripotency

* Please attach a Material Safety Data Sheet or equivalent.

*These modifications will be carried out by the Ontario iPSC Facility, Toronto <http://www.ontarioipsc.ca/index.html>

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? YES NO *OK*

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 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 6.0

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

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

5.3 How will the biological agent be administered? _____

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

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

6.0 Animal Experiments

6.1 Will live animals be used? 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 in section 4.0 be used in live animals YES, specify: _____ NO

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

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

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
If NO, please forward the permit to the Biosafety Officer when available.

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 U.S.A.
If no, please proceed to Section 12.0 NO

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO
- not required

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

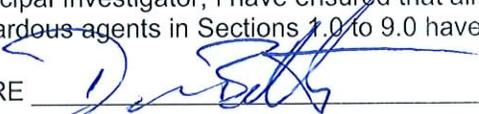
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

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  _____

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

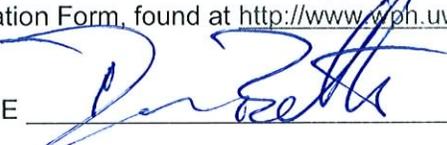
13.0 Containment Levels

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

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus _____
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

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

SIGNATURE  Date: Nov. 30, 2009

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

N/A

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

- as per UWO biosafety guidelines: wash exposed area after allowing the wound to bleed freely; Supervisor/Principal Investigator must be informed of the exposure incident; prompt medical attention must be sought, taking any information including the Material Safety Data Sheet or equivalent; an Accident/Incident Report must be filled out and submitted to OHS.

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

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

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

Special Conditions of Approval:

Subject: Re: Fwd: Biohazardous Agents Registry Form: Betts

From: Dean Betts <Dean.Betts@schulich.uwo.ca>

Date: Fri, 08 Jan 2010 11:10:01 -0500

To: Jennifer Stanley <jstanle2@uwo.ca>

CC: astanki2@uwo.ca, hmulholl@uwo.ca

Hi Jennifer,

I've forwarded your email to Toronto. They suppose to have started the viral infection there, so it will be a few months before the cells arrive here. These cell should be level II, but the future level III+ lab is currently under construction.

We are probably good for a level 2 inspection in DSB 2019 next wed. The biological safety cabinets were moved, installed and certified yesterday and the gas tanks brackets are being installed today. We'll get further organized and be ready for an inspection next week.

Thanks

Dean

Dean H. Betts
Associate Professor
Department of Physiology & Pharmacology
Department of Obstetrics and Gynaecology
Schulich School of Medicine & Dentistry
Medical Sciences Building, Room M 207
The University of Western Ontario
London, Ontario, Canada
N6A 5C1

Office: 519-661-3786
Fax: 519-661-3927
Lab: 519-661-2111 ext. 88090

dean.betts@schulich.uwo.ca

Website: <http://www.physpharm.fmd.uwo.ca/department/faculty/BettsD.html>

||| Jennifer Stanley <jstanle2@uwo.ca> 01/07/10 3:26 PM >>> |||

Hi Dr. Betts:

Based on the viral vector policy (attached), this project is Level 2 with level 3 procedures (level 2+) unless you have evidence such as PCR or other data, that shows that there is no viral shedding. Do you (or your colleagues in Toronto) have data on this?

Thanks
Jennifer

----- Original Message -----
Subject: Biohazardous Agents Registry Form: Betts
Date: Wed, 23 Dec 2009 17:31:23 -0500
From: Jennifer Stanley <jstanle2@uwo.ca>
To: Dean Betts <Dean.Betts@schulich.uwo.ca>
CC: avpres@uwo.ca <avpres@uwo.ca>

Hi Dr. Betts

Thank you for your recent submission. The project will be Level 2 plus unless you can show that there is no viral shedding - do you know if the facility in Toronto has this data?

I have attached our Viral Vector policy for your review.

Happy Holidays,
Jennifer

University of Western Ontario Biohazardous Agents Registry Form
Project Description: Dean H. Betts, Dept. Physiology & Pharmacology

Epigenetic Regulation of telomere length using an X-autosome chromosome model

Telomeres are the native capping structures at the end of chromosomes, composed of hexameric DNA repeats (TTAGGG)ⁿ and proteins that play a pivotal role in chromosome stability, cell cycle regulation, cellular aging and the silencing of adjacent genes. Telomeres gradually shorten with every cell division in most somatic cells due to the end-replication problem of unidirectional DNA synthesis. In contrast, most immortalized cells, including germ cells maintain their telomere length by activating telomerase; a ribonucleic protein that adds telomeric repeats onto the chromosome ends using an RNA component as a template. Telomere length is adjusted during meiosis followed by a phase of telomere elongation during preimplantation embryo development. The mechanism by which telomere length is regulated is complex, with a body of evidence suggesting the involvement of telomerase-dependent and telomerase-independent mechanisms including homologous recombination between telomeres; genetic factors; epigenetic mechanisms such as DNA methylation and in the case of the X-chromosome, sex differences. In particular, the epigenetic process of X-chromosome inactivation in females whereby one of the two X-chromosomes becomes hypermethylated and hypoacetylated in response to a cis-acting non-coding RNA is associated with extensive chromatin remodeling. This is not unlike the mechanism proposed for the silencing of genes of the subtelomeric regions by cis acting telomeres transcripts. Interestingly, this heterochromatinization process differentially affects telomere length regulation of the inactivated and activated X-chromosomes.

Experimental evidence now indicates that chromatin modifications are important regulators of telomere structure and function. Recent studies suggest a role of telomere-binding proteins (TBPs) and RNAs (the shelterin complex) in maintaining telomere length homeostasis and interacting with other heterochromatin regions such as the inactive X-chromosome. We, and others, have shown that drastically shortened telomeres from various donor somatic cells derived from aged animals or near-senescent cell cultures can be rebuilt to shorter, normal or even longer lengths by somatic cell nuclear transfer (SCNT). The extensive reprogramming that occurs during SCNT offers a novel experimental tool in the study of control of telomere homeostasis, with the active and inactive X chromosomes providing homologous chromosomes one of which has highly methylated subtelomeric regions.

Hypothesis: Epigenetic processes modulate telomere length changes and facilitate chromosome-specific telomere length regulation during early development and in pluripotent stem cells.

Specific objectives:

1. To determine the role of the epigenetic profile of telomeric and subtelomeric regions and the mechanism(s) of telomere length dynamics during early development and pluripotent stem cells.

2. To alter various epigenetic regulators to investigate telomerase-dependent and independent mechanisms of global telomere length homeostasis.
3. To delineate the telomerase-dependent and telomerase-independent mechanisms of chromosome-specific telomere length changes during development and in pluripotent stem cells.

Research Plan: The experiments outlined here will examine the role(s) of distinct telomere structure in regulating telomere length dynamics during early embryo development using a unique X-autosome chromosome model. We will examine the expression and reprogramming of key genes involved in chromatin remodeling at telomeric and sub-telomeric repeats and elucidate the role of specific epigenetic modifications in this process. Cloning by SCNT and induction of pluripotency (iPS), techniques proven capable of reprogramming differentiated somatic cell nuclei into pluripotent cells, and gene-silencing by RNA *interference*, will be used as bioassays to examine the effects of altering telomere and subtelomere chromatin modifications on telomerase activity, telomere length, developmental potential, pluripotency and self-renewal. Specific probes for chromosome immunoprecipitation (ChIP); quantitative Q-FISH, RNA-FISH, serial immuno-FISH and Real Time PCR will be applied to bovine embryos and embryonic stem (ES)-like cell lines produced by SCNT of female donor bovine somatic cells containing an X-autosome translocation or to induced pluripotent stem cells (iPSC) generated from human fibroblasts containing an X-autosome translocation by retroviral and lentiviral induction of pluripotency factors (**work initially conducted by the Ontario Human Induced Pluripotent Stem Cell Facility, Toronto**). In addition, loss of function studies of TBPs would be conducted using pharmacological inhibition and RNA interference and gain of function studies using expression vector systems would be undertaken. Morphological and cellular changes that ensue following the adoption of these techniques monitored using standard molecular techniques. These studies will further our understanding of telomere length control during early embryo development. Understanding the epigenetic mechanisms by which telomere length and other heterochromatin regions are regulated is fundamental for understanding telomere biology, fetal origins of adult diseases and other telomere-related diseases, such as cancer and aging.

Institute of Aging and
Reproductive Health

Institute of Cancer
Research

Institute of Circulation
and Respiratory Health

Institute of Gender and
Health

Institute of Genetics

Institute of Health Services
and Policy Research

Institute of Aging

Institute of Human
Development and Child
and Youth Health

Institute of Infection
and Immunity

Institute of Musculoskeletal
Health and Arthritis

Institute of Neurosciences,
Mental Health and Addiction

Institute of Nutrition,
Metabolism and Diabetes

Institute of Population and
Public Health

Institut de la santé
des Autochtones

Institut du cancer

Institut de la santé
circulatoire et respiratoire

Institut de la santé des
femmes et des hommes

Institut de génétique

Institut des services et
des politiques de la santé

Institut du vieillissement

Institut du développement
et de la santé des enfants
et des adolescents

Institut des maladies
infectieuses et immunitaires

Institut de l'appareil
locomoteur et de l'arthrite

Institut des neurosciences,
de la santé mentale et
des toxicomanies

Institut de la nutrition,
du métabolisme et du diabète

Institut de la santé publique
et des populations

July 21, 2009

Dr. Dean BETTS
Department of Physiology & Pharmacology
Medical Sciences Building, Rm M207
The University of Western Ontario
London, Ontario
N6A 5C1

Re: Stem Cell Oversight Committee review of Children's Health Research Institute-funded project "Chemical Induction of pluripotent stem cells from human umbilical cord blood stem cells"

Dear Dr. Betts:

Thank you for your clarification of the nature of the in-kind provision of the cell lines, requested in the letter of July 9, 2009. SCOC made a number of recommendations that were reviewed and accepted by CIHR's Governing Council in July. The Committee recommended that your request to use the SCOC-approved CA1, CA2, H9 human embryonic stem cell lines and induced human pluripotent stem cells for this proposal be approved.

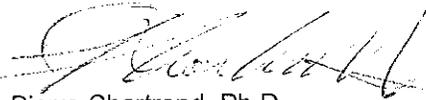
This letter constitutes final approval by CIHR of the above-mentioned project for the use of human embryonic stem cell lines CA1, CA2 and H9 and induced human pluripotent stem cells. As CIHR does not notify co-applicants, we ask that you please inform those individuals and their research institutions (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, Manager – Program Planning and Analysis



Public Health
Agency of Canada

Agence de santé
publique du Canada

Protected when Completed
Protéger une fois rempli

Centre for Emergency Preparedness
and Response

Centre de mesures et d'interventions d'urgence

**Application for permit to
import human pathogen(s)**

**Demande de permis d'importation d'un
(d')agent(s) anthropopathogène(s)**

**For Office use only
À l'usage du bureau seulement**

Under the authority of the Human
Pathogens Importation Regulations.

Sous le régime du Règlement sur l'importation des
agents anthropopathogènes.

Permit no. - N° de permis

Forward copy to:
Office of Laboratory Security
100 Colonnade Road, Loc.: 6201A
OTTAWA ON K1A 0K9
Telephone: (613) 957-1779 Facsimile: (613) 941-0596

Envoyer la copie au :
Bureau de la sécurité des laboratoires
100, chemin Colonnade, Loc. : 6201A
OTTAWA ON K1A 0K9
Téléphone : (613) 957-1779 Télécopieur : (613) 941-0596

1. Applicant - Name, address and postal code / Demandeur - Nom, adresse, et code postal Dr. Dean H. Betts Dept. Physiology & Pharmacology, University of Western Ontario, London, ON, N6A 5C1	Facsimile Télécopieur 519-661-3827	Telephone no. N° de téléphone 519-661-3786
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2. Supplier - Name and address / Fournisseur - Nom et adresse
**Coriell Cell Repositories, 403 Haddon Avenue
Camden, New Jersey 08103, USA**

3. Description of material comprising human pathogen (Including name of material, country of origin and human or animal source)
Description de la matière comprenant un agent anthropopathogène (notamment dénomination, pays d'origine et source humaine ou animale)

Established Human Fibroblast cell lines

4. Mode of transportation Mode de transport Courier truck/van (FedEx)	5. Canadian port(s) of entry (Note: Not more than one entry is permissible in the case of a human pathogen that belongs to risk group 3 or 4.) Point(s) d'entrée au Canada (Remarque : Une seule entrée est permise dans le cas d'un agent anthropopathogène des groupes de risque 3 ou 4.)
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6. Quantity of material to be imported and in the case of a human pathogen belonging to risk group 2, any intervals at which, or period during which, the pathogens are to be imported.
Quantité de la matière à importer - Dans le cas d'un agent anthropopathogène du groupe de risque 2, toute intervalle ou période d'importation.
5 cell culture flasks of live, established human fibroblast cell lines deemed not to be contaminated or infected with biohazardous agents

7. Description of applicant's facilities and equipment for handling material (Note: Appropriate containment is required: see the LABORATORY BIOSAFETY GUIDELINES as amended from time to time, established by Health Canada and the Medical Research Council of Canada).
Description des installations et de l'équipement du demandeur utilisés pour la manutention de la matière (Remarque : Confinement adéquat exigé : voir les LIGNES DIRECTRICES EN MATIÈRE DE BIOSÉCURITÉ EN LABORATOIRE, avec leurs modifications successives, établies par Santé Canada et le Conseil de recherche médicales du Canada).

Appropriate human cell culture facilities and cryopreservation equipment is present and available at the applicant's facilities (University of Western Ontario).

Additional information attached Yes No
Renseignements complémentaires ci-joint Oui Non

8. Address of location where the human pathogen is to be used / Adresse du lieu où sera utilisé l'agent anthropopathogène
Medical Sciences Building, Schulich School of Medicine & Dentistry, UWO, London

9. Method of treatment of material for the purposes of decontamination, sterilization and waste disposal
Méthode de traitement de la matière aux fins de décontamination, de stérilisation et de l'élimination des déchets
Cells or material exposed to human cells will be decontaminated by autoclave sterilization/incineration.

10. Work objectives, proposed plan of work and additional pertinent information / Objectifs des travaux, plan de travail proposé et autres renseignements utiles
These cell lines will by culture propagated and analyzed for epigenetic regulation of X-inactivation status and telomere length.

11. Applicant
I undertake that the material comprising the human pathogen will, in the event of its importation, be used in accordance with such terms and conditions as may be specified in the permit, and I certify that the facilities in which the material will, in that event, be manipulated and stored meet the following containment level;

Demandeur
Je m'engage à ce que la matière comprenant l'agent anthropopathogène, dans l'éventualité de son importation, soit utilisée conformément aux conditions du permis d'importation et j'atteste que les installations dans lesquelles cette matière sera manipulée et entreposée satisfont aux exigences du niveau de confinement suivant;

Containment level (Check one block only)
Niveau de confinement (Ne cocher qu'une seule case)
 1 2 3 4

Signature of applicant - Signature du demandeur

Date
Nov. 27, 2009

*Note:
Physical containment levels and mechanical systems, operational protocols and laboratory waste disposal facilities are subject to verification as may be required by the Director.

*Remarque :
Les niveaux de confinement physique ainsi que les systèmes mécaniques, les protocoles opérationnels et les installations d'élimination des déchets de laboratoire sont soumis à une vérification si le Directeur l'exige.

STATEMENT OF STATUS OF MATERIAL INTENDED FOR IMPORT

1. Description of material intended for import (including supplier name/address):

Established human fibroblast cell cultures deemed not to be contaminated or infected with biohazardous agents from Coriell Cell Repositories, 403 Haddon Avenue, Camden, New Jersey 08103, U.S.A.

phone: 1-856-757-4848

Fax: 1-856-757-9737

2. I, Dr. Dean H. Betts, the undersigned, have reasonable grounds to believe that the material described in 1. does not contain human immunodeficiency virus type 1 and/or 2, human T-cell lymphotropic virus type 1 and/or 2, hepatitis viruses (A, B, C), the agent causing syphilis (*Treponema pallidum*), or any other human pathogen.



Signature

Nov. 27, 2009

Date

CORIELL CELL REPOSITORIES

Coriell Institute for Medical Research
403 Haddon Avenue
Camden, NJ 08103

1-800-752-3805 (USA)

1-856-757-4848 (other countries)

1-856-757-9737 FAX

E MAIL: ccr@coriell.org
jpeluse@coriell.org

INTERNET: NIGMS Catalog - <http://ccr.coriell.org/>
NIA Catalog - <http://ccr.coriell.org/>

If you have placed an order with us and are following up with further forms or information, mention that your order is in progress and provide the P.O. number *and reference number assigned to your order*.

Date: November 24, 2009

To: University of Western Ontario, Purchasing Dept.

P.O. #: 524867

FAX: 519 661 3772

From: Judy Peluse
Coriell Cell Repositories

Message: Thank you for your recent order for biomaterials from the Coriell Cell Repositories. Your order cannot be processed as submitted as we no longer accept paper orders (faxed in or emailed) after March 1, 2009.

We are now only accepting orders using our online catalog system at <http://ccr.coriell.org>. The process involves the following:

- Create a user name (email address) and password to log into our system
- Register all the vital information for a new online account including Principal Investigator, Billing Address, Shipping Address, and Institutional Official (previous customer information including shipping history and assurance forms will be connected to the new electronic registration during the Coriell approval process)
- You will receive an automated email in your mailbox that will give quick instructions on how to activate your account
- You can begin putting items in a shopping cart list and the list can be saved at any time for future reference

FAX Cover Sheet: JP

- You can "check out" your shopping cart after your account has been verified by Coriell Customer Service and your order will now be placed with Coriell. You will receive a four digit Pre-Order Number and your order can now be printed out for your records.
- The rest of the process remains the same as before- all submitted orders will be reviewed by the Coriell Order Review Board and you will receive emails as the order is processed through the system

*****Purchase Orders generated at your company can still be used for payment- you will be prompted to submit this information during the checkout process of your "shopping cart".

Detailed instructions for creating a new account can be seen at <http://ccr.coriell.org/Sections/Support/Global/FirstOrder.aspx?PgId=499>.

Please direct all inquires about online registration and ordering to Arlene Carlton at acarlton@coriell.org or at 856-757-9697. Our Customer Service Representatives will continue to be available for shipping and product information at ccr@coriell.org or 800-752-3805 in USA (856-757-4848 from other countries).

NIGMS Human Genetic Cell Repository

Assurance Form for Human Cell Lines, Somatic Cell Hybrids, and DNA Samples

Revised Version, July 25, 2006

To ensure compliance with the Office for Human Research Protections (OHRP), Department of Health and Human Services (DHHS), regulations for the protection of human subjects (45 CFR Part 46), before human cell cultures or DNA samples can be shipped from the NIGMS Human Genetic Cell Repository, the principal investigator must provide the Repository with a written description of the purpose of the research to be done using the cell cultures or DNA samples. Both the principal investigator and the institutional official who is authorized to make legally binding agreements for the institution must sign this statement agreeing to adhere to the following conditions.

The written description of research purpose and the signed Assurance Form must be returned to the Coriell Cell Repositories.

WARRANTY AND LIABILITY

The recipient acknowledges that the conditions for use of the research materials (cell cultures and DNA samples) are governed by the NIGMS Human Genetic Cell Repository Institutional Review Board (IRB) in accordance with DHHS regulations (45 CFR Part 46). The recipient agrees to comply fully with all such conditions and to report promptly to the NIGMS Human Genetic Cell Repository IRB any proposed changes in the research project and any unanticipated problems involving risks to subjects or others. The recipient remains subject to all applicable state and local laws or regulations and institutional policies which provide additional protections for human subjects.

Repository staff will under no circumstances provide information that will allow investigators to identify subjects. Furthermore, the recipient agrees not to try to identify or contact the submitter of the sample or the donor subject from whom the cell line or DNA sample was derived.

The recipient also agrees not to name the population from whom the samples were obtained, if this information is not essential. (See Policy for the Responsible Collection, Storage, and Research Use of Samples from Identified Populations for the NIGMS Human Genetic Cell Repository).

Warranty: THE REPOSITORY MAKES NO REPRESENTATIONS AND EXTENDS NO WARRANTIES OF ANY KIND, EITHER EXPRESSED OR IMPLIED. THERE ARE NO EXPRESS OR IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, OR THAT THE USE OF THE MATERIAL WILL NOT INFRINGE ANY PATENT, COPYRIGHT, TRADEMARK, OR OTHER PROPRIETARY RIGHTS.

Liability Statement for State Institutions: The recipient institution agrees to be responsible for

any claims, costs, damages, or expenses resulting from any injury (including death), damage, or loss that may arise from the use of the cell culture or DNA sample to the extent permitted under the laws of the recipient's state. This provision shall also apply to any byproducts or derivative of the cell cultures or DNA samples.

Liability Statement for U.S. Government Laboratories: The United States assumes the liability for any claims, damages, injuries, or expenses arising from the use of material or any byproduct or derivative, but only to the extent provided under the Federal Tort Claims Act (28 U.S.C. Chapter 171).

Liability Statement for All Other Institutions: The recipient institution agrees to indemnify and hold harmless the United States Government, Coriell Institute for Medical Research, and the contributor from any claims, costs, damages, or expenses resulting from any injury (including death), damage, or loss that may arise from the use of the cell culture or DNA sample. This provision shall also apply to any byproducts or derivatives of the cell culture or DNA sample.

HUMAN EXPERIMENTATION

Human experimentation utilizing the research materials (cell cultures and DNA samples or their derivatives) may not be undertaken without additional prior review and approval by the NIGMS Human Genetic Cell Repository IRB and by an IRB at the recipient site, which must be convened under an applicable OHRP-approved Assurance.

RESEARCH USE, COMMERCIAL USE, AND RESTRICTIONS ON REDISTRIBUTION AND PROHIBITIONS ON RESALE

The Coriell Cell Repositories provide biomaterials as a service to the research community. The purpose of the NIGMS Human Genetic Cell Repository is to stimulate and facilitate research in genetics and related fields, leading to a better understanding of normal genetic and cellular processes, to the identification and function of disease-related genes, and to the diagnosis and treatment of genetic disorders.

It is expressly understood that the biomaterials delivered pursuant to this Agreement are experimental and are for use in research, in teaching and as standards in clinical genetics laboratories. Recipients employing cell cultures or DNA samples for use as research standards or controls are responsible for complying with all laws and regulations applicable to the intended use of the materials, including any requirements for FDA approval.

There is no restriction on development of commercial products resulting from the knowledge gained from studies using Repository cell lines or DNA samples. However, the distribution of Repository cell lines or DNA samples, or material isolated from them, in commercial products or services is strictly prohibited.

Other uses of the cell lines and DNA samples, and products derived from them, are subject to the following prohibitions and restrictions. Secondary distribution and shared use of cell cultures and DNA samples (including the expansion or subdivision of cell cultures or replication or subdivision of DNA) or the distribution of products derived from cell cultures or DNA samples obtained from the Human Genetic Cell Repository, with or without charge, is prohibited except under special circumstances (see "Shared Use and Secondary Distribution").

BIOHAZARD

All cultured animal and human cells have the potential for carrying viruses, latent viral genomes, and other infectious agents in a latent or inactive state. The cell cultures shipped by the Repository should therefore not be treated as if they are free of contamination. These cells should always be handled carefully by trained persons under laboratory conditions which afford adequate biohazard containment following MINIMUM SAFETY GUIDELINES RECOMMENDED FOR WORKING WITH HUMAN CELL CULTURES. By accepting these cells, the undersigned assume full responsibility for their safe and appropriate handling.

We, the undersigned, have read and understand this document and agree to adhere to the restrictions and warnings stated therein.

Name of Institution: The University of Western Ontario

Principal Investigator (typed or printed): Dr. Dean H. Betts

Signature: 

Institutional Official who can make legal commitments on behalf of the Institution (typed or printed):

Please see the document regarding the Institutional Official

Title of Institutional Official: _____

Signature of Institutional Official: _____

Date: _____

Version 8.1: July 25, 2006

To contact the CORIELL CELL REPOSITORIES:

Write: 403 Haddon Avenue; Camden, New Jersey 08103 USA

Call: 800-752-3805 in the United States; 856-757-4848 from other countries

Fax: 856-757-9737

e-mail: ccr@coriell.org

**NIGMS HUMAN GENETIC CELL REPOSITORY
STATEMENT OF RESEARCH INTENT**

For each research project submit separate Statements of Research Intent.
Please fill out all parts of the form. Use additional sheets as necessary.

Date: November 23, 2009

Part I: List the Repository number for each cell culture, DNA sample, or DNA Panel you wish to order:

Repository Number	Description
GM04626	47 (X,X,X) - Trisomy X Fibroblasts
GM07693	Translocation t(X;10)Xp11 Fibroblasts
GM00089	Translocation t(X;19)Xq22 Fibroblasts
GM11459	Translocation t(X;3)Xp22 Fibroblasts
GM04628	Translocation t(X;22)Xq12 Fibroblasts

Part II: These samples will be used in the following ways (Check all that apply):

- Perform functional studies
- Develop or characterize induced pluripotent stem cell lines (iPS)
- Serve as positive or negative controls for genetic testing
- Serve as positive or negative controls for assay development
- SNP discovery/Genotyping/haplotyping
- Sequence portions of the genome
- Map genes
- Identify novel genes
- Characterize genes and mutations
- Study gene expression
- Study molecular phylogenies
- Determine the ancestral state of a polymorphism/haplotype
- Conduct proteomic studies
- Other (please specify) Determine X-inactivation status and telomere length

NIGMS HUMAN GENETIC CELL REPOSITORY
STATEMENT OF RESEARCH INTENT
Continued

Part III: Please describe more specifically the study or studies you will conduct using these samples. (You may type and attach the description, or include a copy of the abstract of your research grant that describes the project). If, in the future, you plan to use these samples for a purpose different from what you provide here, you must submit another Statement of Research Intent. There will be no additional charge.

This study will evaluate the epigenetic regulation of X-chromosome telomere length dynamics in X-autosome translocated, and trisomy X human cells lines before and after cellular reprogramming into induced pluripotent stem (iPS) cells. Various X-chromosome gene expression patterns, epigenetic dynamics (including X-inactivation status) and telomere length analyses will be carried out. iPS cell lines will be initially generated by the Ontario Human Induced Pluripotent Stem Cell Facility (Director: James Ellis; Tel: 416-813-7295
E-mail: jellis@sickkids.ca)

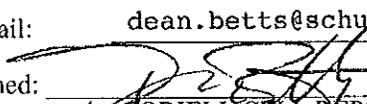
Part IV: Please provide information about proposed secondary distribution, if any.
All shared usage must conform to the Secondary Use Policy.

- These samples will be used only in my laboratory.
- These samples will be shared with one or more investigators for a single research study.
- These samples will be shared as part of a multi-user core facility.
- These samples will be distributed as aliquots or derivatives for use as biological standards.
- These samples will be shared as a unique biological resource.

Part V: Contact information.

Please provide the e-mail address of the scientist directly responsible for the use of the cell culture or DNA sample.

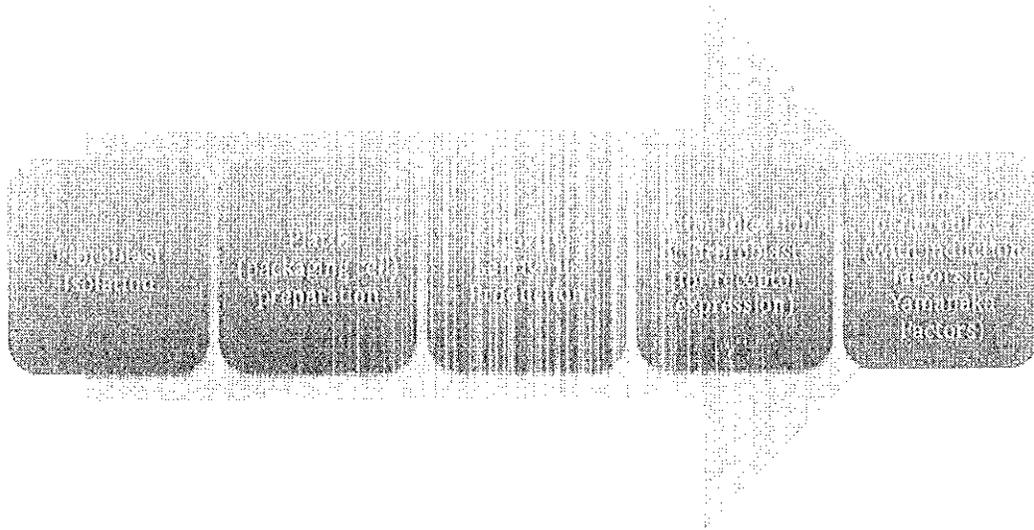
Name: Dean H. Betts
Institution: University of Western Ontario
e-mail: dean.betts@schulich.uwo.ca

Signed: 

To contact the CORIELL CELL REPOSITORIES:

Write: 403 Haddon Avenue; Camden, New Jersey 08103; USA
Call: 800-752-3805 in the United States; 856-757-4848 from other countries
Fax: 856-757-9737 e-mail: ccr@coriell.org

Basic Overall Workflow



Retroviral Vector Production Protocol

Please check reagent and preparation list before continuing

DAY 1: Initial Density-specific Seeding of Cells To Be Infected

1. From an actively growing culture of Plat-E cells count the appropriate number of cells to be seeded for transfection (using a hemocytometer).

Seed cells according to the following chart based on the surface area of the culture dish you will be using.

Dish Type	Surface Area/well or dish (cm ²)	Required Seeding Cell #	Volume of Culture Medium To Use (ml)
24-well	2	2×10^5	0.5
12-well	4.01	4×10^5	1
6-well (35mm)	9.62	1×10^6	2
T-25 (60mm)	25	2.5×10^6	5
T-75 (100mm)	75	7.5×10^6	10
T-160	160	1.6×10^7	20

2. Gently motion the plate in a 'figure 8' motion or in the motion of forming a '+' sign, to ensure even distribution of the cells throughout the plate.
3. Place your seeded cells in the incubator (which is set to 37°C and 5%CO₂) overnight until the following evening.

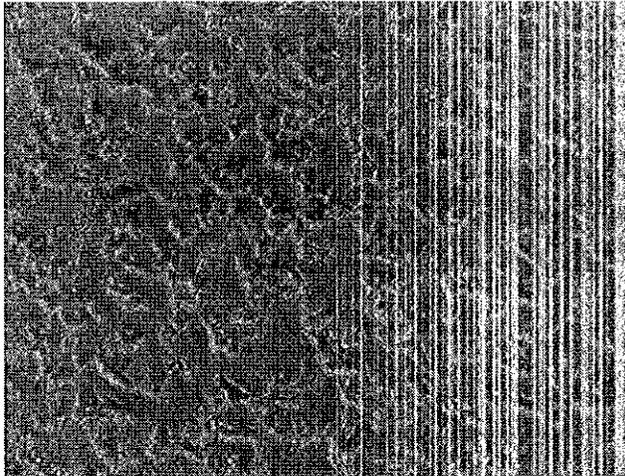


Fig.1 Ideal density of cells on the day of transfection. One day before transfection, 7.5×10^6 cells were seeded into a T-75 flask. *292T (Plat-E)* are shown here

DAY 2: Transfection of Cells With Plasmid Vectors

- Transfection (the process of cells 'up taking' the plasmid) is to be allowed to run 8-16 hours. Thus it is ideal to begin the transfection in the evening and conclude early the following morning.
- There are 2 methods of transfection. Both will be outlined here, however, only one is done per transfection.

Method 1: Calcium Phosphate Transfection (Classical method)

Classical method. Need strict optimization of transfection condition. Reagents are not expensive.

Procedure (for a T-75 flask)

1. Aspirate off old media from Plat-E cells and gently add 10 ml of fresh & warm media into a flask.
2. In a 1.5 ml tube, dilute 30 µg of retroviral vector plasmid in sterilized milli-Q water to make 450 µl of DNA solution and mix with 50 µl of 2.5M CaCl₂. (Total 500 µl of DNA mixture)
3. Prepare a 15 ml Falcon tube with 500 µl of 2xHBS buffer (should be at room temperature) on a stand.
4. Using a 1 ml serological pipette attach to an electronic pipettor in your left hand, make bubbles in the 2xHBS buffer and add dropwise the DNA mixture (from step 1) slowly onto the 2xHBS buffer by using a P1000 Pipetman in your right hand. This mixing step is critical to make close-grained DNA precipitations. Incubation time after mixing is not required.
5. Add dropwise the 1 ml of complexes into a T-75 flask containing cells and medium. Mix gently by rocking the flask back and forth.
6. Incubate cells at 37°C in a CO₂ incubator. Media may be changed after 12 hours.

Method 2: Lipofection using Lipofectamine 2000 (Recommended method)

Easy and highly reproducible method, although reagent is expensive.

1. Mix 30 µg of retroviral vector plasmid with 1.5 ml of Opti-MEM I Reduced Serum Medium (without serum).
2. Dilute 60 µl of Lipofectamine 2000 in 1.5 ml of Opti-MEM® I Medium. Incubate for 3-5 minutes at room temperature.

3. After the incubation, combine the diluted DNA with diluted Lipofectamine 2000 (total volume = 3 ml). Mix gently and incubate for 20 minutes at room temperature (Note: Complexes are stable for 6 hours at room temperature).
4. During the incubation, aspirate off old media of Plat-E cells and gently add 10 ml of fresh & warm medium into a flask.
5. Add dropwise the 3 ml of complexes into a T-75 flask containing cells and media. Mix gently by rocking the flask back and forth.
6. Incubate cells at 37°C in a CO₂ incubator. Media may be changed after 4-6 hours.

DAY 3: Media Change

1. In the morning (12–16 hours after transfection), aspirate the old media and add fresh & warm media into each flask/well.
2. Place the replace the plate back into the same incubator and leave overnight.

DAY 4: Virus Harvesting Using The Ultracentrifuge

1. Cool the rotor in fridge and turn on the centrifuge machine. Close the cover and press "Vacuum" to start cooling. (Login: 1571, PIN: Lab ext)
2. Transfer the virus from the culture plate to a 10 cm dish using a 10 ml pipette.
25 ml pipettes are thick and will scratch the bottom. If you see floating cells, it's better to pre-filter the virus by 40 µm or 100 µm mesh filter because it will clog the filter.
3. Aspirate the virus using a 30 ml syringe and attach a 0.45 µm syringe filter.
4. Filter the virus into a 50 ml tube.
Filtering is required to remove cell debris.
5. Put 20-22 ml of the virus into an ultracentrifuge tube. Typically, 21 ml into a tube.

Save some virus to check the titer before concentration. You MUST fill the tubes to more than 90% of capacity; otherwise, the tubes will break under the high centrifugal forces.

6. Balance the tubes by adding the virus, media or HBSS within 0.1 gram.

Caution! Breaking the rotor and spreading the virus is the most terrible disaster. Always check the balance properly and carefully.

7. Set the tubes into the chilled rotor. Use grease to seal the rotor cover if necessary.
8. Centrifuge 30,000 rpm, 2 hours, 4 degree (Accel: 9, Decel: 7) for VSV-G pseudotyped retrovirus, and 9,100 rpm, 16 hours, 4 degree (Accel: 9, Decel: 7) for ecotropic retrovirus.

Centrifuge speed needs to be adjusted for each rotor size. Since centrifugal efficiency (centrifuge force) depends on the radius from the center, you need to take the size of the tube into account. For example, the SORVALL T-865 rotor has 910 mm maximum radius on the bottom of tube and 384 mm minimum radius at the top of tube. If you run the rotor at 30,000 rpm, the bottom of tube will take $91,482 \times g$ and the top of tube will take only $38,604 \times g$. In my experience, VSV-G pseudotyped lentiviral vector can stand for up to $100,000 \times g$.

Ecotropic envelope is physically weaker than VSV-G envelope. To concentrate ecotropic virus, you need lower speed but longer time (overnight) of centrifugation.

- i. Press "Vacuum" to release the vacuum and open the cover.
 - ii. Open the cover and set the rotor properly and quickly to keep cool inside.
 - iii. Shut the cover and press "Vacuum" immediately.
 - iv. Wait 5-10 min until vacuum and cooling is done.
 - v. Press "Start" to start running.
9. After running, carefully transfer the tubes NOT TO DISTURB the virus pellet. Remember, the virus pellet is fragile and easy to detach from the tube. Aspirate the supernatant immediately after the running.
 10. CAREFULLY remove the whole supernatant using a 10 ml plastic pipette (not 25 ml pipette). The last 1 ml can be removed with a 1 ml plastic pipette. The last 1 ml may contain a certain amount of virus. Spare some for titration if desired

(specially for your 1st experiment).

11. Add chilled 50 μ l HBSS (or media, TAE, etc.) to soak the virus pellet and incubate overnight at 4 degrees.
12. Target cells to be infected need to be seeded at a density that gives 30-40% confluence on the day of infection and will become 80-90% confluent 2 days after infection.
13. Incubate the cells overnight at 37°C and 5% CO₂ in preparation for infection the following day.

DAY 5: Infection of Target Cells By Produced Virus

14. Resuspend the virus pellet by pipetting at least 20 times. Avoid making bubbles because this makes it difficult to collect the virus. Typically, you will get approximately 80 μ l of concentrated virus solution (50 μ l HBSS + left over media from the wall of tube).
15. Change the medium of target cells and add polybrene to a final concentration of 8 μ g/ml
16. Add the virus with several 10-fold dilutions. Do not forget the mock infection.

For precise calculation of the viral titer, trypsinize one of the well and count the cell number (optional).

- For unconcentrated virus, try 100 μ l, 10 μ l, and 1 μ l of virus. For concentrated virus, try 1 μ l, 0.1 μ l, 0.01 μ l of virus. To get these dilutions, put 1 μ l of virus into 500 μ l of media and mix, then simply transfer 50 μ l of mixture into a next well.

17. Incubate the virus containing plate overnight to allow for infection.

DAY 6: Media Change

18. After overnight infection, change the medium to remove virus and polybrene.

DAY 7: GFP Expression Analysis

19. Analyze GFP expression by microscope or flow cytometry.

Since integrated vectors are only a few copies, GFP fluorescence under microscope is much dimmer than that of plasmid transfection. You may need to change the media with PBS and observe in a dark room. I recommend use of flow cytometry to check the GFP%.

Reagent List

- **Plat-E growth media**

DMEM (high glc) [Invitrogen, 11965-092]	500ml
Fetal Bovine Serum (FBS) [Invitrogen, 16000-044, \$403.85]	50ml
Penicillin-Streptomycin-Glutamine [Invitrogen, 10378-016]	5ml

Pass through filter in tissue culture hood and store at 4°C.

- **0.25% Trypsin-EDTA (0.25% Trypsin, EDTA-4Na)** [Invitrogen, 25200-56, 100ml]

Thaw at 4°C overnight and store at 4°C. Minimize warming up to prevent self-inactivation.

- **PBS (Phosphate-Buffered Salines) (pH=7.4)** [Invitrogen, 10010-023, 500ml]
Store at 4°C.

- **Penicillin-Streptomycin-Glutamine** [Invitrogen, 10378-016, 100ml]

Thaw at 4°C overnight and store at 4°C. Minimize warming up to prevent self-inactivation. Added to prevent bacterial contamination, but if you have a good sterilization technique, it's not required. Supplement of L-glutamine helps cell growth and virus production little bit, because L-glutamine is the least stable amino acids. Not essential.

- **Geneticin** [Invitrogen, 10131-035, 20ml] (For SV40 large T antigen selection)

Aliquot and store at -20°C. A working tube can be kept at 4°C.

- **Blasticidin S HCl** [Invitrogen, R210-01, 50 mg] (For MoMLV gag/pol selection)

$C_{17}H_{26}N_8O_5 \cdot HCl$, FW=458.9,

Make 10 mg/ml stock solution in water. Aliquot and store at -20°C.

A working tube can be kept at 4°C. Final concentration in culture medium is 10 mg/ml (x1000).

- **Puromycin dihydrochloride** [Sigma, P8833, 10mg] (For MoMLV env selection)

Aliquot and store at -20°C. A working tube can be kept at 4°C.
Make 20 mg/ml stock solution in water. Prepare 1 mg/ml concentration as for a working solution. Final concentration in culture medium is 1 mg/ml (x1000).

Transfection Reagents

Calcium Phosphate Transfection (Classical Method)

- **2.5M CaCl₂ (For 25 ml)**

CaCl ₂ anhydrous (FW=111) [Sigma, C-4901]	6.94g
Milli-Q water	up to 25ml

Pass through filter in tissue culture hood and store at -20°C.

- **2x HBS (For 100 ml)**

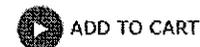
NaCl (FW=58.44) [FisherScientific, S271-1]	1.6g
KCl (FW=74.55) [BDH, B29594]	0.074g
Na ₂ HPO ₄ anhydrous (FW=141.96) [BDH, ACS807]	0.0213g
D-(+)-Glucose (FW=180.2) [Sigma, G-7528]	0.2g
HEPES (FW=238.3) [Sigma, H-7006]	1.0g
Milli-Q water	approx. 80ml

NOTE: pH of 2xHBS is critical for high efficiency transfection. Adjust the pH before and after adding H₂O (Remember, pH of solution can be shifted by adding H₂O). You may need to optimize the pH of HBS by making several range of pH (such as, 7.00, 7.05, and 7.10).

- pH of HEPES buffer is temperature dependent. (1°C of increase gives 0.03 decrease of pH)
- pH of HBS should be adjusted at room temperature. Bring the HBS to room temperature before use.

Lipofection using Lipofectamine 2000 (recommended method)

- **Lipofectamine™ 2000** Transfection Reagent [Invitrogen, 11668-019, 1.5ml]
- **Opti-MEM I Reduced-Serum Medium** [Invitrogen, 31985-062, 100ml]

Catalog ID: **GM04628**Product (Source): **CELL CULTURE**

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- [Characterizations](#)
- [Phenotypic Data](#)
- [Publications](#)
- [External Links](#)
- [Images](#)
- [Protocols](#)

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Chromosome Abnormalities
Sample Description TRANSLOCATED CHROMOSOME
Cell Type Fibroblast
Transformant Untransformed
Species Homo sapiens
Common Name Human
Sex Female
Ethnicity SWISS
Relation to Proband proband
Confirmation Karyotypic analysis after cell line submission to CCR
ISCN 46,X,t(X;22)(Xpter>Xq12::22p11>22pter; 22qter>22p11::Xq12>Xqter)
Remarks Normal X is late replicating

Catalog ID GM04628
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00

How to Order [Online Ordering](#)
[Assurance Form](#) (Must have current form on file)
[Statement of Research Intent Form](#) (Information will be entered electronically when order is placed. DO NOT fax form to Coriell Customer Service)

Characterizations

Sample Description TRANSLOCATED CHROMOSOME
Passage Frozen 3

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Chromosome Analysis

Cytogenetics Chromosome 22: TRANSLOCATION Breakpoint 22p11 t(X;22)22p11
 Chromosome X: TRANSLOCATION Breakpoint Xq12 t(X;22)Xq12

Phenotypic Data

Remark Normal X is late replicating

Publications

Carrel L, Willard HF, Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. Proc Natl Acad Sci U S A 96:7364-9 1999

PubMed ID: [10377420](#)

Lafreniere RG, Brown CJ, Powers VE, Carrel L, Davies KE, Barker DF, Willard HF, Physical mapping of 60 DNA markers in the p21.1----q21.3 region of the human X chromosome. Genomics 11:352-63 1991

PubMed ID: [1685139](#)

Buhler E, Clinical and cytological aspects of sex chromosome activity. Hereditas 86:63-74 1977

PubMed ID: [903252](#)

Buhler EM, Jurik LP, Voyame M, Buhler UK, Presumptive evidence of two active X chromosomes in somatic cells of a human female. Nature 265:142-4 1977

PubMed ID: [834254](#)

External Links

dbSNP [dbSNP ID: 20964](#)

Images

Data are not available

Protocols

Passage Frozen 3

Split Ratio 1:2

Temperature 37 C

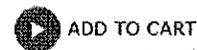
Percent CO2 5%

Medium Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids

Serum 15% fetal bovine serum Not inactivated

Substrate None specified

Subcultivation Method trypsin-EDTA

Catalog ID: **GM11459**Product (Source): **CELL CULTURE**

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- [Publications](#)
- [External Links](#)
- [Images](#)
- [Protocols](#)

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Chromosome Abnormalities
Sample Description TRANSLOCATED CHROMOSOME
Biopsy Source Embryo
Cell Type Fibroblast
Tissue Type Whole embryo
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 18 FW
Sex Female
Relation to Proband proband
Confirmation Clinical summary/Case history
ISCN 46,X,t(X;3)(Xqter>Xp22.1::3q23> 3qter;3pter>3q23::Xp22.1>Xpter)
Remarks Product of conception fibroblast culture

Catalog ID GM11459
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00

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Characterizations

Sample Description TRANSLOCATED CHROMOSOME
Passage Frozen 3

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Nucleoside Phosphorylase, Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase Isoenzyme Electrophoresis and by Chromosome Analysis

Cytogenetics Chromosome X: TRANSLOCATION Breakpoint Xp22 t(X;3)Xp22

Phenotypic Data

Remark Product of conception fibroblast culture

Publications

Carrel L, Willard HF, Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. Proc Natl Acad Sci U S A 96:7364-9 1999

PubMed ID: [10377420](#)

External Links

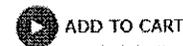
dbSNP [dbSNP ID: 22554](#)

Images

Data are not available

Protocols

Passage Frozen 3**Split Ratio** 1:5**Temperature** 37 C**Percent CO2** 5%**Medium** Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids**Serum** 15% fetal bovine serum Not inactivated**Substrate** None specified**Subcultivation Method** trypsin-EDTA

Catalog ID: **GM00089**Product (Source): **CELL CULTURE**

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- [Publications](#)
- [External Links](#)
- [Images](#)
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Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Chromosome Abnormalities
Sample Description TRANSLOCATED CHROMOSOME
Cell Type Fibroblast
Transformant Untransformed
Species Homo sapiens
Common Name Human
Sex Female
Race Caucasian
Family [1236](#)
Family Member 1
Relation to Proband proband
Confirmation Karyotypic analysis after cell line submission to CCR
ISCN 46,X,t(X;19)(Xpter>Xq22::19q13.3>19qter; 19pter>19q13.3::Xq22>Xqter)
Remarks Normal X is late replicating

Catalog ID GM00089

Product Cell Culture

Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00

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Characterizations

Sample Description TRANSLOCATED CHROMOSOME
Passage Frozen 5

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Chromosome Analysis

Cytogenetics Chromosome 19: TRANSLOCATION Breakpoint 19q13 t(X;19)19q13
 Chromosome X: TRANSLOCATION Breakpoint Xq22 t(X;19)Xq22

Phenotypic Data

Remark Normal X is late replicating

Publications

Carrel L, Willard HF, Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. *Proc Natl Acad Sci U S A* 96:7364-9 1999

PubMed ID: [10377420](#)

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PubMed ID: [2889662](#)

Oberle I, Camerino G, Wrogemann K, Arveiler B, Hanauer A, Raimondi E, Mandel JL, Multipoint genetic mapping of the Xq26-q28 region in families with fragile X mental retardation and in normal families reveals tight linkage of markers in q26-q27. *Hum Genet*77:60-5 1987

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Oberle I, Camerino G, Kloepfer C, Moisan JP, Grzeschik KH, Hellkuhl B, Hors-Cayla MC, Van Cong N, Weil D, Mandel JL, Characterization of a set of X-linked sequences and of a panel of somatic cell hybrids useful for the regional mapping of the human X chromosome. *Hum Genet*72:43-9 1986

PubMed ID: [3002952](#)

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PubMed ID: [2986139](#)

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PubMed ID: [752537](#)

External Links

dbSNP [dbSNP ID: 13947](#)

Images

Data are not available

Protocols

Passage Frozen 5

Split Ratio 1:3

Temperature 37 C

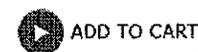
Percent CO2 5%

Medium Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids

Serum 15% fetal bovine serum Not inactivated

Substrate None specified

Subcultivation Method trypsin-EDTA

Catalog ID: **GM04626**Product (Source): 

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- [Phenotypic Data](#)
- [Publications](#)
- [External Links](#)
- [Images](#)
- [Protocols](#)

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Chromosome Abnormalities
Sample Description ANEUPLOID CHROMOSOME NUMBER - TRISOMY
Cell Type Fibroblast
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 21 FW
Sex Female
Race Caucasian
Relation to Proband proband
Confirmation Karyotypic analysis after cell line submission to CCR
ISCN 47,XXX[49]/46,XX[1]
Remarks Abortus; clinically normal phenotype

Catalog ID GM04626
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00

Catalog ID NA04626
Product DNA
Quantity 0.050mg
Source cell culture
Pricing Commercial Pricing: \$55.00
 Academic and not-for-profit pricing: \$55.00

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Characterizations

Sample Description ANEUPLOID CHROMOSOME NUMBER - TRISOMY
Passage Frozen 5

IDENTIFICATION OF SPECIES OF ORIGIN Species of Origin Confirmed by Chromosome Analysis

GENE MAPPING & DOSAGE STUDIES - Y CHROMOSOME PCR analysis of DNA from this cell culture gave a negative result with a primer for Yq11, DYS227.

Cytogenetics Chromosome X: ANEUPLOID Aneuploid Segment (+)Xpter>Xqter

Phenotypic Data

Remark Abortus; clinically normal phenotype

Publications

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Ensminger AW, Chess A, Coordinated replication timing of monoallelically expressed genes along human autosomes. Hum Mol Genet13(6):651-8 2004

PubMed ID: [14734625](#)

Lage JM, Leamon JH, Pejovic T, Hamann S, Lacey M, Dillon D, Segraves R, Vossbrinck B, Gonzalez A, Pinkel D, Albertson DG, Costa J, Lizardi PM, Whole genome analysis of genetic alterations in small DNA samples using hyperbranched strand displacement amplification and array-CGH. Genome Res13(2):294-307 2003

PubMed ID: [12566408](#)

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins C, Kuo WL, Chen C, Zhai Y, Dairkee SH, Ljung BM, Gray JW, Albertson DG, High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. Nat Genet20:207-11 1998

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Kallioniemi A, Kallioniemi OP, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D, Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science258:818-21 1992

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PubMed ID: [2390975](#)

External Links

dbSNP [dbSNP ID: 10746](#)

GEO [GEO Accession No: GSM282120](#)

[GEO Accession No: GSM282142](#)

[GEO Accession No: GSM282147](#)

[GEO Accession No: GSM282343](#)

[GEO Accession No: GSM282354](#)

[GEO Accession No: GSM282359](#)

Images

View [karyotype](#)

Protocols

Passage Frozen 5

Split Ratio 1:5

Temperature 37 C

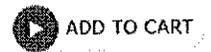
Percent CO2 5%

Medium Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids

Serum 10% fetal bovine serum Not inactivated

Substrate None specified

Subcultivation Method trypsin-EDTA

Catalog ID: **GM07693**Product (Source): **CELL CULTURE**

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Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Chromosome Abnormalities
Sample Description TRANSLOCATED CHROMOSOME
Biopsy Source Placenta
Cell Type Fibroblast
Tissue Type Placental
Transformant Untransformed
Species Homo sapiens
Common Name Human
Sex Female
Race Caucasian
Relation to Proband proband
Confirmation Karyotypic analysis after cell line submission to CCR
ISCN 46,X,t(X;10)(Xqter>Xp11.2::10q24.3> 10qter;10pter>10q24.3::Xp11.2>Xpter)
Remarks Placenta biopsy; loose skin, scoliosis, small thorax, scaphoid abdomen, hypoplastic labia, right clinodactyly and camptodactyly, thenar hypoplasia, left camptodactyly, diaphragm hernia, pulmonary hypoplasia, bicornuate uterus, and absent right olfactory lobe

Catalog ID GM07693
Product Cell Culture
Pricing Commercial Pricing: \$85.00
 Academic and not-for-profit pricing: \$85.00
How to Order [Online Ordering](#)
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Characterizations

Sample Description TRANSLOCATED CHROMOSOME
Passage Frozen 4

IDENTIFICATION OF SPECIES Species of Origin Confirmed by Nucleoside Phosphorylase, Glucose-6-Phosphate Dehydrogenase, and Lactate Dehydrogenase
OF ORIGIN Isoenzyme Electrophoresis and by Chromosome Analysis

Cytogenetics Chromosome 10: TRANSLOCATION Breakpoint 10q24 t(X;10)10q24
 Chromosome X: TRANSLOCATION Breakpoint Xp11 t(X;10)Xp11

Phenotypic Data

Remark Placenta biopsy; loose skin, scoliosis, small thorax, scaphoid abdomen, hypoplastic labia, right clinodactyly and camptodactyly, thenar hypoplasia, left camptodactyly, diaphragm hernia, pulmonary hypoplasia, bicornuate uterus, and absent right olfactory lobe

Publications

Carrel L, Willard HF, Heterogeneous gene expression from the inactive X chromosome: an X-linked gene that escapes X inactivation in some human cell lines but is inactivated in others. Proc Natl Acad Sci U S A 96:7364-9 1999

PubMed ID: [10377420](#)

External Links

dbSNP [dbSNP ID: 17413](#)

Images

Data are not available

Protocols

Passage Frozen 4**Split Ratio** 1:3**Temperature** 37 C**Percent CO2** 5%**Medium** Eagle's Minimum Essential Medium with Earle's salts and non-essential amino acids**Serum** 15% fetal bovine serum Not inactivated**Substrate** None specified**Subcultivation Method** trypsin-EDTA