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

Location of experimental work to be carried out: Building(s) MSB, DSB Room(s) M298A 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:

Heather Mulholland
Adam Stankiewicz
John Soleas
Lida Radan
Stephanie Hallows

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? O YES Ø 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? O YES O 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
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? Ø YES O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	Ø Yes O No	Foreskin	Not applicable
Rodent	O Yes Ø No		
Non-human primate	O Yes Ø No		
Other (specify)	O Yes Ø 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	⊗ Yes O No	- CA1/CA2/H9 ESCs - Fibroblasts	- Mt Sinai Hospital - Coriell Institute
Rodent	⊗ Yes O No	- mouse embryonic fibroblasts	sick kids hospital
Non-human primate	O Yes ⊗ No		
Other (specify)	O Yes ⊗ No		

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

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

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O 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		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 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 O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O 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 O NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results
VGV-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.ontarioips.ca/index.html

4.4 Will genetic sequences from the following be involved?

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

4.5 Will virus be replication defective? O YES ⊗ NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES ⊗ NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ O NO

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

_____

_____

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES ⊗ NO If no, please proceed to Section 9.0

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

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

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

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES ⊗ NO
If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

*** 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? O 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? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O 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? O YES O NO
If NO, please forward the permit to he 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 O NO

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

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

11.4 Has the import permit been sent to OHS? O 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

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

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

O YES, permit # if on-campus_____
Ø NO, please certify
O 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:

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

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 telomers transcripts. Interestingly, this heterochromatization 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.

CIHR IRSC

Institute of Aboriginal Peoples' Health

Institute of Cancer Research

Institute of Circulatory 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

/lnc-bb-me

Canadian Institutes of Health Research
Room 97, 160 Elgin Street, Address locator: 4809A
Ottawa, Ontario K1A 0W9 Tel.: (613) 941-2672
Fax (613) 954-1800 **www.cihr.ca**

Instituts de recherche en santé du Canada
Pièce 97, 160 rue Elgin, Indice de l'adresse: 4809A
Ottawa (Ontario) K1A 0W9 Tél.: (613) 941-2672
Fax (613) 954-1800 **www.irsc.ca**

Canada

Public Health Agency of Canada / Agence de santé publique du Canada

Centre for Emergency Preparedness and Response / Centre de mesures et d'interventions d'urgence

Protected when Completed
Protéger une fois rempli

Application for permit to Import human pathogen(s)

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

Under the authority of the Human Pathogens Importation Regulations.

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

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

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 / Point(s) d'entrée au Canada (Note: Not more than one entry is permissible in the case of a human pathogen that belongs to risk group 3 or 4.) (Remarque : Une seule entrée est permise dans le cas d'un agent anthropopathogène des groupes de risque 3 ou 4.)

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 / Renseignements complémentaires ci-joint: ☐ Yes / Oui ☒ No / 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.

Canada

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 lymphotrophic 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 Institute for Medical Research	Customer Service Implementation Date 08/06/07	FAX Cover Sheet: JP	Form 1304-01 Rev E

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

- 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).

Physiology and Pharmacology (Phys/Pharm)
Purchase Order Form

Supplier Name:	Phys/Pharm Reference Number:
CORIELL CELL REPOSITORIES	
Supplier Address/Phone Number/Fax Number:	UWO Purchase Order Number:
403 Haddon Avenue Camden, New Jersey 08103 USA Phone: 856-757-4848 FAX: 856-757-9737 e-mail: ccr@coriell.org	Please check here if Radioactive: ☐
	PERMIT #:

Order Date:	Researcher/Lab:	Lab Room Number:	Lab Phone/Extension:	Speed Code:	Account:
NOV. 23, 2009	Betts	MSB 298	X88090	PWGP	622000

Description *(please print clearly)*:	Catalogue Number:	UoM:	Quantity:	Unit Price:
Fibroblast cell culture 47 (X,X,X) -- trisomy X	**GM04626**	**EA**	**1**	**$85 ($US)**
Fibroblast cell culture – X- autosome translocation t(X;10)Xp11	**GM07693**	**EA**	**1**	**$85 ($US)**
Fibroblast cell culture – X- autosome translocation t(X;19)Xq22	**GM00089**	**EA**	**1**	**$85 ($US)**
Fibroblast cell culture – X- autosome translocation t(X;3)Xp22	**GM11459**	**EA**	**1**	**$85 ($US)**
Fibroblast cell culture – X- autosome translocation t(X;22)Xq12	**GM04628**	**EA**	**1**	**$85 ($US)**

Please indicate UoM (Unit of Measure): EA, PKG, BOX, DZN, CASE, KIT, PAIR

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
- [X] 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
- [X] Study gene expression
- [ ] Study molecular phylogenies
- [ ] Determine the ancestral state of a polymorphism/haplotype
- [ ] Conduct proteomic studies
- [X] 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.
- [x] 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

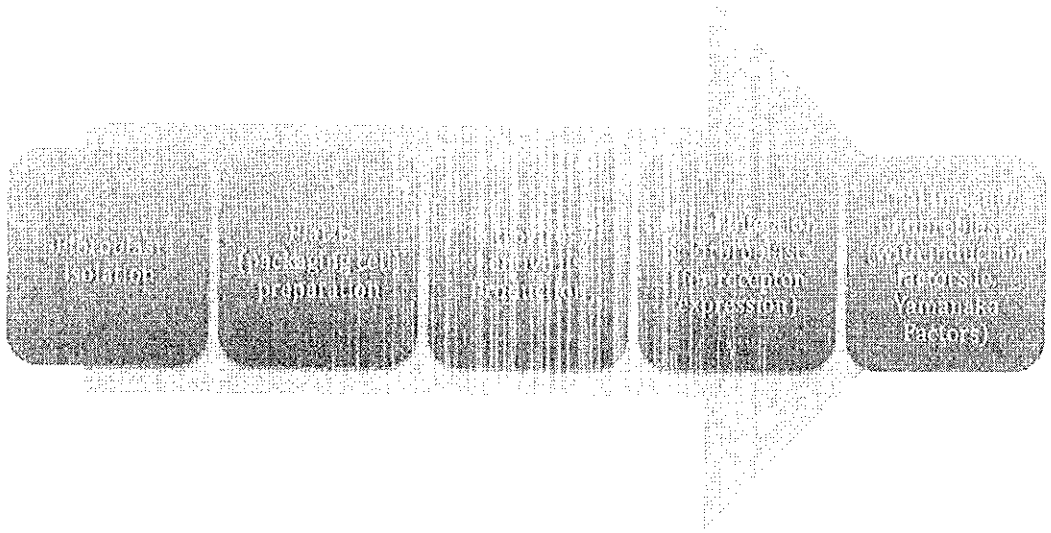
Institution: University of Western Ontario

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	$2x10^5$	0.5
12-well	4.01	$4x10^5$	1
6-well (35mm)	9.62	$1x10^6$	2
T-25 (60mm)	25	$2.5x10^6$	5
T-75 (100mm)	75	$7.5x10^6$	10
T-160	160	$1.6x10^7$	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%CO_2) overnight until the following evening.

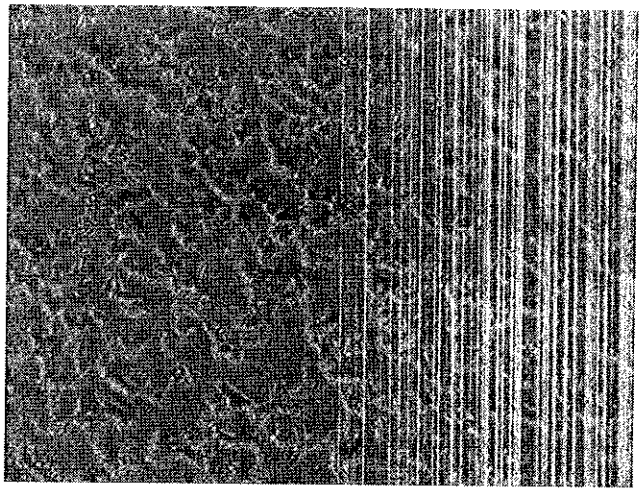


Fig.1 Ideal density of cells on the day of transfection. One day before transfection, $7.5x10^6$ cells were seeded into a T-75 flask. *292T (Plat-E) are shown here*

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 $CaCl_2$. (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 CO_2 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 CO_2 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 um 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 $x\,g$ and the top of tube will take only 38,604 $x\,g$. In my experience, VSV-G pseudotyped lentiviral vector can stand for up to 100,000 $x\,g$.

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 µl 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% CO_2 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 µl of concentrated virus solution (50 µl 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 µg/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 µl, 10 µl, and 1 µl of virus. For concentrated virus, try 1 µl, 0.1 µl, 0.01 µl of virus. To get these dilutions, put 1 µl of virus into 500 µl of media and mix, then simply transfer 50 µl 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 many 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 [Invitorgen, 10378-016] 5ml

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

- **0.25% Trypsin-EDTA (0.25% Trypsin, EDTA-4Na)** [Invitorgen, 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)** [Invitorgen, 10010-023, 500ml]
  Store at 4˚C.

- **Penicillin-Streptomycin-Glutamine** [Invitorgen, 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** [Invitorgen, 10131-035, 20ml] (For SV40 large T antigen selection)

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

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

  $C_{17}H_{26}N_8O_5$-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 4ºC. 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 4ºC.
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 $CaCl_2$ (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_2HPO_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 H_2O (Remember, pH of solution can be shifted by adding H_2O). 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 ADD TO CART

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 A96:7364-9 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. Genomics11:352-63 1991
PubMed ID: 1685139

Buhler E, Clinical and cytological aspects of sex chromosome activity. Hereditas86:63-74 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. Nature265:142-4 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 ADD TO CART

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
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 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 A96:7364-9 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 ADD TO CART

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
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 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 A96:7364-9 1999
PubMed ID: 10377420

Tsukada S, Saffran DC, Rawlings DJ, Parolini O, Allen RC, Klisak I, Sparkes RS, Kubagawa H, Mohandas T, Quan S, et al, Deficient expression of a B cell cytoplasmic tyrosine kinase in human X- linked agammaglobulinemia. Cell72:279-90 1993
PubMed ID: 8425221

Berchtold MW, Koller M, Egli R, Rhyner JA, Hameister H, Strehler EE, Localization of the intronless gene coding for calmodulin-like protein CLP to human chromosome 10p13-ter. Hum Genet90:496-500 1993
PubMed ID: 8428750

Couillin P, Mollicone R, Grisard MC, Gibaud A, Ravise N, Feingold J, Oriol R, Chromosome 11q localization of one of the three expected genes for the human alpha-3-fucosyltransferases, by somatic hybridization. Cytogenet Cell Genet56:108-11 1991
PubMed ID: 1672847

Smeets H, Bachinski L, Coerwinkel M, Schepens J, Hoeijmakers J, van Duin M, Grzeschik KH, Weber CA, de Jong P, Siciliano MJ, et al, A long-range restriction map of the human chromosome 19q13 region: close physical linkage between CKMM and the ERCC1 and ERCC2 genes. Am J Hum Genet46:492-501 1990
PubMed ID: 2309701

Schonk D, van Dijk P, Riegmann P, Trapman J, Holm C, Willcocks TC, Sillekens P, van Venrooij W, Wimmer E, Geurts van Kessel A, et al, Assignment of seven genes to distinct intervals on the midportion of human chromosome 19q surrounding the myotonic dystrophy gene region. Cytogenet Cell Genet54:15-9 1990
PubMed ID: 1701111

Holm C, Kirchgessner TG, Svenson KL, Fredrikson G, Nilsson S, Miller CG, Shively JE, Heinzmann C, Sparkes RS, Mohandas T, et al, Hormone-sensitive lipase: sequence, expression, and chromosomal localization to 19 cent-q13.3. Science241:1503-6 1988
PubMed ID: 3420405

Noguchi T, Mattei MG, Oberle I, Planche J, Imbert J, Pelassy C, Birg F, Birnbaum D, Localization of the mcf.2 transforming sequence to the X chromosome. EMBO J6:1301-7 1987
PubMed ID: 3038515

Arveiler B, Oberle I, Mandel JL, Genetic mapping of nine DNA markers in the q11----q22 region of the human X chromosome. Genomics1:60-6 1987
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 Genet77:60-5 1987
PubMed ID: 3502701

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 Genet72:43-9 1986
PubMed ID: 3002952

Oberle I, Drayna D, Camerino G, White R, Mandel JL, The telomeric region of the human X chromosome long arm: presence of a highly polymorphic DNA marker and analysis of recombination frequency. Proc Natl Acad Sci U S A82:2824-8 1985
PubMed ID: 2986139

Dracopoli NC, Rettig WJ, Albino AP, Esposito D, Archidiacono N, Rocchi M, Siniscalco M, Old LJ, Genes controlling gp25/30 cell-surface molecules map to chromosomes X and Y and escape X-inactivation. Am J Hum Genet37:199-207 1985
PubMed ID: 4038849

Geldwerth D, Bishop C, Guellaen G, Koenig M, Vergnaud G, Mandel JL, Weissenbach J, Extensive DNA sequence homologies between the human Y and the long arm of the X chromosome. EMBO J4:1739-43 1985
PubMed ID: 4029125

Camerino G, Grzeschik KH, Jaye M, De La Salle H, Tolstoshev P, Lecocq JP, Heilig R, Mandel JL, Regional localization on the human X chromosome and polymorphism of the coagulation factor IX gene (hemophilia B locus). Proc Natl Acad Sci U S A81:498-502 1984
PubMed ID: 6320191

Mohandas T, Sparkes RS, Hellkuhl B, Grzeschik KH, Shapiro LJ, Expression of an X-linked gene from an inactive human X chromosome in mouse-human hybrid cells: further evidence for the noninactivation of the steroid sulfatase locus in man. Proc Natl Acad Sci U S A77:6759-63 1980
PubMed ID: 6935682

Hellkuhl B, Grzeschik KH, Partial reactivation of a human inactive X chromosome in human-mouse somatic cell hybrids. Cytogenet Cell Genet22:527-30 1978
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): ---Select One---

ADD TO CART

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
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 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

Isaksson M, Stenberg J, Dahl F, Thuresson AC, Bondeson ML, Nilsson M, MLGA--a rapid and cost-efficient assay for gene copy-number analysis Nucleic acids research35:e115 2007
PubMed ID: 17823203

Warshawsky I, Chernova OB, Hübner CA, Stindl R, Henneke M, Gal A, Natowicz MR, Multiplex ligation-dependent probe amplification for rapid detection of

proteolipid protein 1 gene duplications and deletions in affected males and carrier females with Pelizaeus-Merzbacher disease Clinical chemistry52:1267-75 2006
PubMed ID: 16644873

Peiffer DA, Le JM, Steemers FJ, Chang W, Jenniges T, Garcia F, Haden K, Li J, Shaw CA, Belmont J, Cheung SW, Shen RM, Barker DL, Gunderson KL, High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping Genome research16:1136-48 2006
PubMed ID: 16899659

Wang Y, Moorhead M, Karlin-Neumann G, Falkowski M, Chen C, Siddiqui F, Davis RW, Willis TD, Faham M, Allele quantification using molecular inversion probes (MIP) Nucleic acids research33:e183 2005
PubMed ID: 16314297

Garcia MJ, Pole JC, Chin SF, Teschendorff A, Naderi A, Ozdag H, Vias M, Kranjac T, Subkhankulova T, Paish C, Ellis I, Brenton JD, Edwards PA, Caldas C, A 1 Mb minimal amplicon at 8p11-12 in breast cancer identifies new candidate oncogenes Oncogene24:5235-45 2005
PubMed ID: 15897872

Barrett MT, Scheffer A, Ben-Dor A, Sampas N, Lipson D, Kincaid R, Tsang P, Curry B, Baird K, Meltzer PS, Yakhini Z, Bruhn L, Laderman S, Comparative genomic hybridization using oligonucleotide microarrays and total genomic DNA. Proc Natl Acad Sci U S A101(51):17765-70 2004
PubMed ID: 15591353

Bignell GR, Huang J, Greshock J, Watt S, Butler A, West S, Grigorova M, Jones KW, Wei W, Stratton MR, Futreal PA, Weber B, Shapero MH, Wooster R, High-resolution analysis of DNA copy number using oligonucleotide microarrays. Genome Res14(2):287-95 2004
PubMed ID: 14762065

Zhao X, Li C, Paez JG, Chin K, Janne PA, Chen TH, Girard L, Minna J, Christiani D, Leo C, Gray JW, Sellers WR, Meyerson M, An integrated view of copy number and allelic alterations in the cancer genome using single nucleotide polymorphism arrays. Cancer Res64(9):3060-71 2004
PubMed ID: 15126342

Ota A, Tagawa H, Karnan S, Tsuzuki S, Karpas A, Kira S, Yoshida Y, Seto M, Identification and characterization of a novel gene, C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. Cancer Res64(9):3087-95 2004
PubMed ID: 15126345

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
PubMed ID: 9771718

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
PubMed ID: 1359641

Zacksenhaus E, Sheinin R, Molecular cloning, primary structure and expression of the human X linked A1S9 gene cDNA which complements the ts A1S9 mouse L cell defect in DNA replication. EMBO J9:2923-9 1990
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 ADD TO CART

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
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 4

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 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 A96:7364-9 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