THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: March 27, 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 ~~also~~ be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, 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: Dr. Robert Hegele
SIGNATURE:
DEPARTMENT: Robarts Research Institute, Vascular Biology Research Group
ADDRESS: 100 Perth Dr. Room 4-06
PHONE NUMBER: 519-931-5271 Lab. 519-931-5777 ext. 34412
EMERGENCY PHONE NUMBER(S): 519-931-5271
EMAIL: hegele@robarts.ca

Location of experimental work to be carried out: Building(s) RRI Room(s) 4288, 4292, 4286

*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: HSFO, CIHR
GRANT TITLE(S):

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

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

Jian Wang
Henian Cao
Brooke Kennedy
John Robinson
Reina Hassell
Rebecc Provost
Matthew Ban
Chris Johansen
Piya Lahiry
Matthew Oaktree

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☒YES O NO
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O 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
DH 10B	O Yes ☒No	O Yes ☒No	O Yes ☒No	0.007-0.010	Invitrogen	☒1 O2 O3
DH 5α	O Yes ☒No	O Yes ☒No	O Yes ☒No	0.007-0.010	"	☒1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3
	O Yes O No	O Yes O No	O Yes O No			O1 O2 O3

*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 O NO
If no, please proceed to Section 3.0

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒Yes O No	See Attached	Not applicable
Rodent	☒Yes O No	" "	"
Non-human primate	O Yes ☒No		
Other (specify)	O Yes ☒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 the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	☒ Yes ○ No	See Attached	See Attached
Rodent	☒ Yes ○ No	" "	ATCC
Non-human primate	☒ Yes ○ No	COS-7	ATCC
Other (specify)	○ 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 ○ 1 ☒ 2 ○ 3

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	Research Subjects	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Blood (fraction) or other Body Fluid	Research Subjects	○ Yes ☒ No		○ 1 ☒ 2 ○ 3
Human Organs or Tissues (unpreserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3
Human Organs or Tissues (preserved)		○ Yes ○ No		○ 1 ○ 2 ○ 3

4.0 Genetically Modified Organisms and Cell lines

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

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
DH5α	pcDNA 3.1 CAT	Bacteria (Invitrogen)	APOE	Morphological

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV 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 ☒YES O NO
- E1A oncogene ☒YES WILL B ~~☒~~NO
- Known oncogenes O YES, please specify _____ ☒NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ ☒NO

4.5 Will virus be replication defective? ☒YES O 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 using the viral vector in 4.0? O YES ☒NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

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

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

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

8.5 How much of the toxin is stored*? _____

*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 lifestage of the insect? _____

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

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*? _____

*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 lifestage of the insect? _____

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

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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. O 1 ☒ 2 O 3

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

☒ YES, permit # if on-campus BIO-RR1-0006

O 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 [signature] Date: Apr. 24. 2009

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: [signature]
Date: May 07, 2009

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***

2009 Biohazard Agents Registry, Dr. Robert Hegele

Name	CAT. NO.	SUPPLIER	MSDS	Comment	Use
3T3-L1	CL-173	ATCC	Y	Murine fibroblast	Mutant LMNA is transfected into these cells and immunofluorescence studies and Western analysis performed
NIH/3T3	CRL-1658	ATCC	Y	Murine	
COS-7: note SV40 promoter	CRL-1651	ATCC	Y	Green monkey (Ceropithecus aethiops)	
HEK293: note Adeno E1A promoter	CRL-1573	ATCC	Y	Human	
HepG2	CRL-11997	ATCC	Y	Human	
Fibroblast, finite primary cell line human	GM05659	Coriell Cell Repository	Y	Human: unaffected	Cells are cultured, morphology and growth curves evaluated and immunofluorescence studies, Western analysis, DNA and RNA isolated and microarray and high throughput sequencing analysis performed.
Fibroblast, finite primary cell line human	GM08398	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	GM03348	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG03513	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG04456	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	AG06234	Coriell Cell Repository	Y	Human: HGPS proband	
Fibroblast, finite primary cell line human	AG06297	Coriell Cell Repository	Y	Human: unaffected	
Fibroblast, finite primary cell line human	30950	Dr. T.C. Rupar	na	Human	Cells are cultured, DNA and RNA extracted and microarray and high throughtput sequencing analysis performed
Fibroblast, finite primary cell line human	40916	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	20750	Dr. T.C. Rupar	na	Human	
Fibroblast, finite primary cell line human	70280	Dr. T.C. Rupar	na	Human	
pCMV6	PS100001	Origene	y		Transfection vectors used in the lab
pcDNA3.1	350492	Invitrogen	Y		
pENTR 11 dual selection vector	A10562	Invitrogen	Y		
Subcloning Efficiency™ DH5a™ Competent Cells	18265-017	Invitrogen	Y	E. Coli	Cells used to as vehicles for transfection of genetic material for overexpression studies and phenotypic characterization
Electromax DH10B competent cells	18290-015	Invitrogen		E. Coli	
ME DH10B competent cells	18297-010	Invitrogen		E. Coli	
ME DH5-alpha competent cells	18258-012	Invitrogen	Y	E. Coli	
ME DH5A T1 page resist comp cells	12034-013	Invitrogen		E. Coli	

Public Health Agency of Canada | Agence de la santé publique du Canada

Your file / Votre référence

Our file / Notre référence

Name and/or Organization: **University of Western Ontario**
Robarts Research Institute
Attn: Dr. Robert Hegele

Address: P.O. Box 5015, 100 Perth Drive
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell lines from healthy donor (AG06234) and from donor with Hutchinson-Gilford progeria syndrome (AG06297), as provided by Coriell Institute for Medical Research, 403 Haddon Avenue, Camden, NJ, USA 08103.

Marianne Heisz
Chief, Importation and Regulatory Affairs

APRIL 20, 2009
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

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

Your file *Votre référence*

Our file *Notre référence*

Date issued: October 4, 2006

Name and/or Organization: **Robarts Research Institute**
Attn: Dr. Robert A. Hegele

Address: 100 Perth Drive, Room 4-06
London, ON
N6A 5K8

The following biological material does not require a Public Health Agency of Canada import permit under the HPIR*:

Human fibroblast cell lines from healthy donor (AG04456) and from donor with Hutchinson-Gilford Progeria Syndrome (AG03513), as provided by Coriell Cell Repositories, NJ, USA.

Paul J. Payette, Ph.D.
Director, Office of Laboratory Security

October 4, 2006
Date

NOTICE

***HPIR (HUMAN PATHOGENS IMPORTATION REGULATIONS)**

- We are in receipt of your application for an importation permit for biological materials. The **HPIR** apply **only** to the importation of infectious substances which cause human disease and their subsequent distribution or transfer. Other materials, which are deemed by the importer to be non-infectious for humans, **do not** require a permit under these regulations. It should be noted that the importation of biological materials may also be subject to other federal, provincial and municipal laws.
- For animal or plant pathogens one **must** apply to The Canadian Food Inspection Agency (CFIA) for a permit to import. If this material is of animal or plant origin it may also require a permit from the CFIA. Please contact the CFIA for their consideration. CFIA contact numbers are as follows:
  (613) 221-7068 for information concerning animal pathogens/material
  (613) 225-2342 [ext. 4334] for information concerning plant pathogens/material
- Importation of this material may also be subject to the requirements of the *New Substances Notification Regulations (Organisms)* of the *Canadian Environmental Protection Act, 1999*, administered by Environment Canada and Health Canada. Please contact the New Substances Information Line at 1-800-567-1999 or nsn-infoline@ec.gc.ca.
- You may be required to provide the Canada Border Services Agency (CBSA) customs officers with a declaration that the imported material is non-infectious and non-hazardous.

Should you require further information, please contact:
Office of Laboratory Security
Centre for Emergency Preparedness and Response
(613) 957-1779

Canada

CORIELL INSTITUTE FOR MEDICAL RESEARCH

403 Haddon Avenue
Camden,New Jersey 08103
(800) 752-3805 in the U.S.A.
(856)757-4848 from other Countries
FAX (856) 757-9737

Invoice No.:	44305
Invoice Date:	11/1/2006 3:02:13 PM
Ship Date:	10/30/2006

CIMR FEDERAL I.D.#21-0672684
AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

Bill To: 14582

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ROBARTS RESEARCH INSTITUTE
DOCK 50/ VASC BIOL RES GRP
100 PERTH DRIVE RM 4-06
LONDON, ON N6A 5K8 CANADA

Ship to: 14582

ROBERT HEGELE
ROBARTS RESEARCH INSTITUTE
DOCK 50/ VASC BIOL RES GRP
100 PERTH DRIVE RM 4-06
LONDON, ON N6A 5K8 CANADA

ORDER#	PURCHASE ORDER#	CREDIT CARD#	SHIPPED VIA	F.O.B	TERMS
54810	5001-846-417		FedEx	CAMDEN,NJ	NET 30 DAYS

NIA & NIGMS REPOSITORIES

PURCHASER AGREES THAT CELLS,THEIR PROGENY OR DERIVATIEVES WILL NOT BE USED FOR HUMAN EXPERIMENTATION UNLESS PRIOR WRITTEN APPROVAL IS OBTAINED AND THAT OWNERSHIP OF CELL LINES RESIDES WITH THE U.S.GOVERNMENT OR THE ORIGINAL DEVELOPER AND THAT THE CELLS SHALL NOT BE SOLD FOR COMMERCIAL PURPOSES NOR DISTRIBUTED TO OTHER PARTIES FOR THOSE PURPOSES. THEY ARE PROVIDED WITHOUT WARRANTY OF FITNESS FOR PARTICULAR PURPOSE OR ANY OTHER WARRANTY EXPRESSED OR IMPLIED, IN ADDITION, RECIPIENTS AGREE TO INDEMNIFY AND HOLD HARMLESS THE UNITED STATES GOVERNMENT, CIMR,AND THE CONTRIBUTOR FROM ANY CLAIMS, COSTS, DAMAGES, OR EXPENSES RESULTING FROM ANY INJURY, DEATH,DAMAGE, OR LOSS THAT MAY ARISE FROM THE USE OF A CELL LINE.

OTHER COLLECTIONS

CONDITIONS OF PURCHASE ARE THE SAME AS ABOVE EXCEPT 'THE SPONSOR OF THE COLLECTIONS ' SHOULD BE SUBSTITUTED FOR 'THE UNITED STATES GOVERNMENT'.

QTY	REPOSITORY #	CODE	UNIT PRICE	AMOUNT
1	AG03513	1 (AG)	$85.00	$85.00

27 Nov 2006

5001-846-417

INVOICE AMOUNT	SHIPPING CHARGES	SPECIAL HANDLING	PREPAYMENT AMOUNT	PRICE ADJUSTMENT	TOTAL	AMOUNT PAID	TOTAL DUE
$85.00	$0.00	$0.00	$0.00	$0.00	$85.00	$0.00	**$85.00**

COPY OF INVOICE MUST BE RETURNED WITH REMITTANCE FOR PROPER CREDIT.
MAKE CHECKS PAYABLE TO 'CORIELL INSTITUTE FOR MEDICAL RESEARCH'.
U.S. CURRENCY ONLY

CORIELL INSTITUTE FOR MEDICAL RESEARCH

403 Haddon Avenue
Camden,New Jersey 08103
(800) 752-3805 in the U.S.A.
(856)757-4848 from other Countries
FAX (856) 757-9737

Invoice No.:	44381
Invoice Date:	11/13/2006 3:02:56 PM
Ship Date:	11/13/2006

CIMR FEDERAL I.D.#21-0672684
AN EQUAL OPPORTUNITY/AFFIRMATIVE ACTION EMPLOYER

Bill To: 14582

ROBERT HEGELE
ROBARTS RESEARCH INSTITUTE
DOCK 50/ VASC BIOL RES GRP
100 PERTH DRIVE RM 4-06
LONDON, ON N6A 5K8 CANADA

Ship to: 14582

ROBERT HEGELE
ROBARTS RESEARCH INSTITUTE
DOCK 50/ VASC BIOL RES GRP
100 PERTH DRIVE RM 4-06
LONDON, ON N6A 5K8 CANADA

ORDER#	PURCHASE ORDER#	CREDIT CARD#	SHIPPED VIA	F.O.B	TERMS
55094	5001-846-417		FedEx	CAMDEN,NJ	NET 30 DAYS

NIA & NIGMS REPOSITORIES

PURCHASER AGREES THAT CELLS,THEIR PROGENY OR DERIVATIEVES WILL NOT BE USED FOR HUMAN EXPERIMENTATION UNLESS PRIOR WRITTEN APPROVAL IS OBTAINED AND THAT OWNERSHIP OF CELL LINES RESIDES WITH THE U.S.GOVERNMENT OR THE ORIGINAL DEVELOPER AND THAT THE CELLS SHALL NOT BE SOLD FOR COMMERCIAL PURPOSES NOR DISTRIBUTED TO OTHER PARTIES FOR THOSE PURPOSES. THEY ARE PROVIDED WITHOUT WARRANTY OF FITNESS FOR PARTICULAR PURPOSE OR ANY OTHER WARRANTY EXPRESSED OR IMPLIED, IN ADDITION, RECIPIENTS AGREE TO INDEMNIFY AND HOLD HARMLESS THE UNITED STATES GOVERNMENT, CIMR,AND THE CONTRIBUTOR FROM ANY CLAIMS, COSTS, DAMAGES, OR EXPENSES RESULTING FROM ANY INJURY, DEATH,DAMAGE, OR LOSS THAT MAY ARISE FROM THE USE OF A CELL LINE.

OTHER COLLECTIONS

CONDITIONS OF PURCHASE ARE THE SAME AS ABOVE EXCEPT 'THE SPONSOR OF THE COLLECTIONS ' SHOULD BE SUBSTITUTED FOR 'THE UNITED STATES GOVERNMENT'.

QTY	REPOSITORY #	CODE	UNIT PRICE	AMOUNT
1	AG04456	1 (AG)	$85.00	$85.00

17 Nov 2006

5001-846-417

INVOICE AMOUNT	SHIPPING CHARGES	SPECIAL HANDLING	PREPAYMENT AMOUNT	PRICE ADJUSTMENT	TOTAL	AMOUNT PAID	TOTAL DUE
$85.00	$0.00	$0.00	$0.00	$0.00	$85.00	$0.00	**$85.00**

COPY OF INVOICE MUST BE RETURNED WITH REMITTANCE FOR PROPER CREDIT.
MAKE CHECKS PAYABLE TO 'CORIELL INSTITUTE FOR MEDICAL RESEARCH'.
U.S. CURRENCY ONLY

→ Paraphrase thesis.
→ Need.

In 2003, we proposed in HSFO #NA5320 to expand understanding of single-gene predisposition to cardiovascular disease (CVD) and since found causative genes and mutations for 3 forms of partial lipodystrophy, namely familial partial lipodystrophies types 2 and 3 (FPLD2, FPLD3) and acquired partial lipodystrophy (APL). Using "phenomics", defined as integrated multidisciplinary research to understand the complex consequences of genomic variation through systematic discovery and cataloguing of standardized phenotypes, we developed standardized, optimized quantitative non-invasive imaging procedures for carotid arteries and adipose stores, all key to this renewal application. The work was recognized by merit awards from several groups, including the American Heart Association in 2004 and the Genetics Society of Canada in 2006. ***We will build on the scientific momentum from the initial funding period with this renewal application.***

• **BACKGROUND AND SIGNIFICANCE:** The constellation of disturbed carbohydrate and insulin metabolism, with central obesity, dyslipidemia (elevated triglycerides [TG] with depressed HDL cholesterol), hypertension, and type 2 DM (T2DM) is called the 'metabolic syndrome' (MetS). Evaluation of patients with extreme monogenic forms of MetS will help us to understand common MetS, just as the study of patients with monogenic dyslipidemias improved understanding and treatment of those diseases. Some monogenic forms of MetS have been molecularly characterized – including those discovered in the Hegele lab - providing important insights and model systems for common MetS.

• **PROPOSED RESEARCH:** We propose to continue to expand our database of lipodystrophy kindreds, to significantly increase our characterization of biochemical, vascular and adipose phenotypes in monogenic MetS and then to assess these new markers in the general population. Specific aims are:

1) To extend our measurements of traditional and non-traditional serum biomarker phenotypes of subjects with FPLD2, FPLD3, APL, other lipodystrophies and familial hypercholesterolemia (FH; positive control for early CVD) and to contrast these according to molecular basis of the disease.
2) To measure baseline non-invasive ultrasound (US) vascular and magnetic resonance imaging (MRI) adipose phenotypes in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH, and to contrast these according to molecular basis of the disease.
3) To serially measure non-invasive US vascular and MRI adipose phenotypes in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH in order to evaluate disease progression, and to contrast these according to molecular basis of the disease.
4) To determine association between risk factors, intermediate phenotypes and atherosclerosis read-outs variables in subjects with FPLD2, FPLD3, APL, other forms of lipodystrophy and FH.

We expect to observe between-genotype differences in phenotypes such as: 1) lipoprotein, metabolic, cytokines, chemokines and other serum biomarkers; 2) carotid IMT at baseline and serial progression; 3) association of traditional and non-traditional risk factors with IMT; and 4) distribution of adipose stores in different forms of lipodystrophy. The presence of novel serum and imaging biomarkers identified in the studies of patients with monogenic MetS will subsequently be evaluated in individuals from the general population with MetS. We expect that some biomarkers that we will identify as being part of the monogenic MetS metabolic signature profile will be translated to the "garden variety" form of MetS.

• **RELEVANCE:** Our past record of finding determinants of CVD risk in Canadian families with monogenic MetS will continue with renewed funding of this project. Our success in finding MetS genetic determinants in pilot studies leads us to expect that this expanded set of samples will provide new insights. MetS is an important new prevalent CVD risk factor, with numerous metabolic abnormalities and a very strong relationship with vascular disease. Better characterization of early phenotypes in monogenic MetS – sensitive "phenomics" – may provide new clues to help solve the puzzle of the common metabolic syndrome. Any between-genotype differences in phenotype severity will help to plan future experiments assessing specific diagnosis and/or treatment.

Name Robert A. Hegele
Nom

5. A complete informative summary of the proposal on this page.
Présentez sur cette page un résumé complet du projet de recherche.

Background: The importance of well-defined CVD phenotypes was highlighted by recent coining of the term "phenomics", defined as integrated multidisciplinary research to understand the complex consequences of genomic variation. Sensitive new phenomic tools can reveal new phenotypic markers, sometimes called "early" or "intermediate" phenotypes. Because well-established "traditional" risk factors do not account for all CVD events, "non-traditional" risk factors have been proposed, including insulin resistance (IR). Evaluation of monogenic IR syndromes might help understand common IR, just as the study of monogenic forms of dyslipidemia, such as familial hypercholesterolemia (FH) improved understanding of those diseases. Some monogenic forms of IR have been molecularly characterized, providing interesting and sometimes important insights. One of these is Dunnigan-type familial partial lipodystrophy (FPLD), which results from mutations in one of two genes: LMNA or PPARG. FPLD is associated with early atherosclerosis end points in Canadian families.

PROPOSED RESEARCH

Study objectives

1) To measure traditional and non-traditional biochemical phenotypes of subjects with FPLD-LMNA, FPLD-PPARG, and FH (positive control monogenic disease with atherosclerosis), and to contrast these according to molecular basis of the disease.
2) To measure baseline non-invasive ultrasound (US) and magnetic resonance imaging (MRI) carotid phenotypes of FPLD-LMNA, FPLD-PPARG and FH subjects, and to contrast these according to molecular basis of the disease.
3) Over three years, to serially measure non-invasive US and MRI vascular (carotid) phenotypes of FPLD-LMNA, FPLD-PPARG, and FH subjects in order to evaluate disease progression, and to contrast these according to molecular basis of the disease.
4) To determine association between risk factors, intermediate phenotypes and atherosclerosis read-outs variables in FPLD-LMNA, FPLD-PPARG, and FH subjects.

Experimental strategy to test for genotype-phenotype associations

Subjects: Patients with FPLD-LMNA (N=40), FPLD-PPARG (N=20), FH (N=30) and matched controls (N=40)
Genotype: DNA sequence determination of *LMNA*, PPARG or *LDLR* as appropriate
Phenotypes: 1) Lipoprotein profile: cholesterol (total, HDL & LDL), TG, apo B & AI, Lp(a); 2) metabolic variables: insulin, glucose, glycosylated hemoglobin (HbA1C), CRP, C-peptide, free fatty acids, adiponectin, leptin, resistin, ASP; 3) Coagulation and inflammatory variables: FBG, PAI-1, TNF-a, IL-6; 4) Carotid US: IMT, TPA, TPV; 5) Carotid MRI: carotid wall volume, plaque composition; 6) Total body MRI: fat redistribution in selected individuals (3 per group)
Hypotheses: We will observe between-genotype differences in: 1) lipoprotein, metabolic, coagulation and inflammatory markers corrected for age, sex and body mass; 2) carotid US and carotid MRI variables; 3) carotid US and MRI plaque progression; 4) association of traditional and non-traditional risk factors with non-invasive vascular read-outs; 5) distribution of adipose stores in FPLD-LMNA versus FPLD-PPARG.

Management plan. Years 1 and 2: Baseline DNA, biochemical and imaging analysis for 130 subjects; Years 2 and 3: 1-year and 2-year follow-up US and MRI for 130 subjects.

Relevance. Monogenic illnesses have provided key insights into CVD risk factors such as hypertension and dyslipidemia. IR is an important newer risk factor, with a very strong relationship with vascular disease. Better characterization of subjects with monogenic IR using sensitive methods – "phenomics" – may provide clues to help solve the puzzle of the common metabolic syndrome.

DESCRIPTION: State the application's broad, long-term objectives and specific aims, making reference to the health relatedness of the project. Describe concisely the research design and methods for achieving these goals. Avoid summaries of past accomplishments and the use of the first person. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from the application. If the application is funded, this description, as is, will become public information. Therefore, do not include proprietary/confidential information. DO NOT EXCEED THE SPACE PROVIDED.

The overall goal of this proposal is to image inflammation in atherosclerosis by developing and testing novel activatable MR imaging agents that can sense myeloperoxidase (MPO) activity in atheromata. Emerging evidence suggests that activated macrophages secrete various enzymes that mediate inflammation in atherosclerosis, and that MPO in particular may be indicative of high-risk ("vulnerable") lesions. A recent clinical trial has further shown that elevated MPO levels strongly predict adverse cardiovascular outcomes in patients with chest pain. It is therefore hypothesized that imaging of local MPO activity will be useful in identifying vulnerable atherosclerotic lesions.

We have recently described two novel approaches for imaging peroxidases using either low molecular weight amplifiable paramagnetic substrates (Mol Imaging 2002;1:16-23) or superparamagnetic enzyme pseudosubstrates (Nanolett 2004;4:119-122). The latter method is based on magnetic relaxation switching (MRSW) a phenomenon that occurs when magnetic nanoparticles are brought into close contact to each other (R2 increase of 4-6 fold with negligible R1 change). These activatable, "smart" agents harness enzyme mediated amplification strategies and can be used to quantitatively measure enzyme activity and inhibition by MR imaging. The current proposal represents an effort to integrate investigators with different expertises to develop, implement and validate the larger field of *enzyme imaging* in atherosclerosis. Together, the team will translate basic results into new methodologies for *in vivo* MR imaging. The ultimate goal of this research is to develop clinically useful imaging tools for the molecular assessment of atherosclerosis in vivo, which are currently limited.

Robert A. Hegele **Genetic determinants of obesity and plasma lipoproteins** $195,937

Summary of Research Proposal

The metabolic syndrome (MetS) affects 25% of Canadian adults and is a potent risk factor for atherosclerosis. Our strategy to understand the common MetS and dyslipidemia is to study rare human monogenic forms, such as familial partial lipodystrophy (FPLD) and familial hypercholesterolemia (FH). We will build on the ongoing record of discovery of causative genes for familial partial lipodystrophy types 2 (FPLD2; MIM 151660) and 3 (FPLD3; MIM 604367) and of human disease mutations causing hyperlipidemia, obesity and diabetes funded by CIHR application #MOP13430.

OBJECTIVE: ***To discover and define additional genetic determinants of obesity and plasma lipoproteins by studying rare monogenic human MetS in Canadian families and communities.***

Goals: 1) Advancing the understanding of FPLD by: a) extending the mutation spectrum in FPLD2 and FPLD3; b) identifying mutations in new FPLD genes; c) defining *in vitro* mechanism(s) of disease of selected mutations; d) extending the FPLD phenotype; defining phenotypic-genotype correlations. **2) Advancing the understanding of FH by:** a) extending the mutation spectrum in FH; b) identifying mutations in new FH genes; c) extending the FH phenotype; defining phenotypic-genotype correlations.

Experimental details: 1a) Extending the mutational spectrum in FPLD2 and FPLD3. Conventional direct sequencing of genomic DNA (gDNA) from 83 FPLD probands showed that 51 had no mutations in genes for FPLD2 [*LMNA* encoding lamin A/C] or FPLD3 [*PPARG* encoding peroxisome proliferator-activated receptor-γ]. The candidate gene approach showed that two patients had novel rare missense mutations in *EMD* encoding emerin. These families will be expanded and evidence for causation will be obtained from co-segregation with phenotype, absence from normal controls and *in vitro* functional assessment. We will determine whether rare *EMD* missense mutations represent a new molecular form of FPLD – "FPLD4". The remaining 49 subjects with no mutation will have these genes re-screened using a new method to detect deletions called MLPA. Some probands with normal MLPA testing of candidate genes may have mutations in new FPLD genes. **1b) Identifying mutations in new FPLD genes.** We will use a candidate gene approach focusing on direct sequence analysis of gDNA. Candidates include genes involved in nuclear envelope biology, such as *LMNB1, LMNB2, LBR, LAP2, NARF, SENP2, MAN1* and *ZMPSTE24/FACE1*, and genes with a functional link to lipodystrophy, such as *RXRA, SREBP1, AGPAT2* and *BSCL2*. Also, some families may be sufficiently informative to perform linkage analysis. **1c)** ***In vitro*** **studies for selected FPLD mutations.** *LMNA* T496FS is the first splicing mutation in FPLD2. We will express the *LMNA* mutant in model cell lines – liver, adipocyte and fibroblast – and will assess effects on cellular phenotype morphologically, including assessment of fat content and assessment of 3-D nuclear structure, co-immunoprecipitation with other nuclear lamin constituents, such as emerin and with mRNA expression profiling. *PPARG* Y355X will be studied *in vitro* collaboratively with Dr. Todd Leff. **1d) Extending the FPLD phenotype.** We will: i) extend novel FPLD3 kindreds with γ4 promoter -14A>G and E1 FS ΔAATG; ii) perform lipidomic, proteomic and transcriptomic comparison of FPLD2 and FPLD3; iii) define the phenotype of pre-symptomatic FPLD children. **2a) Extending the mutational spectrum in FH.** Conventional direct sequencing of gDNA from 109 FH probands showed that 62 had no mutation in the HCHOLAD1 gene [*LDLR*]. We will re-screen these genes from gDNA of affected subjects using MLPA. We will sequence from gDNA of candidate genes including *APOB*, *NARC1/PCSK9* and *ARH*. **2b) New FH genes.** Some FH families have no mutation in a known gene and may be informative for linkage analysis. **2c) Extending the FH phenotype.** We will: 1) extend novel FH families and compare phenotypes between molecular forms.

Relevance. Demonstrating mutant *LMNA* in FPLD introduced a new mechanism - abnormal nuclear envelope structure and function - to the study of MetS and its metabolic complications. Disease mutations can serve as probes for new pathways or mechanisms. The planned studies will also help determine whether haploinsufficiency is an important disease mechanism in FPLD and whether simple replacement with normal gene product is feasible. Mutations in new will specify new pathways, disease mechanisms and therapeutic drug targets.

V SCIENTIFIC SUMMARY (Maximum one (1) page)

Structural and Functional Annotation of the Human Genome for Disease Study

In this application, we plan integrated and comprehensive computational and laboratory-based experiments to produce a more sophisticated and complete structural and functional annotation of human genomic variation, within four major, integrated themes. These themes are: Theme 1 (Scherer): To completely characterize the recently described phenomenon of large-scale copy variations (LCVs) in the human genome; Theme 2 (Hughes and Frey): To elucidate all gene coding sequences; Theme 3 (Blencowe and Frey) To characterize all splicing isoforms of all genes; and Theme 4 (Hegele): To evaluate the role of these newer genomic variations in mechanisms underlying selected human diseases, specifically atherosclerosis, diabetes and breast cancer. Since a complete understanding of genome biology is the ultimate goal of genomic investigation, analysis of patients will play a focal role in increasing our knowledge of function and regulation of the genes and types of variation involved. Our efforts to construct comprehensive new genomic datasets will enhance the characterization of clinical samples, in particular, for monogenic versions of complex diseases such as atherosclerosis, diabetes and breast cancer. Each of the four integrated and complementary themes will use high-throughput technologies within the OGI/Genome Resource Platform to assist in data gathering and will build additional capacity and new infrastructure. State-of-the-art technologies and advanced computational applications that we have developed in recent collaborations will be extended and applied to support each of our four Themes. Importantly, our activities will also be co-ordinated with the Canadian and International scientific community to enhance their application. Partnerships with the Sanger Institute, the European Bioinformatics Institute, the Human Epigenetics Consortium, Affymetrix, Agilent, Rosetta/Merck, and other USA and European genome scientists, have already been established to facilitate the work.

The annotated databases from this project will be made available to the international community, via links to existing genomic resources. They will serve as an international resource for biomedical researchers to address research inquiry into the spectrum of genomic variation in health and disease. The methods and algorithms used to generate the datasets we will also represent intellectual property. Furthermore, the databases will be maintained and curated in Canadian and European centres, in part from support from by private sector co-funds. The addition of the broad range of phenotype data linked to the unprecedented depth of genomic data will permit sophisticated modelling and hypothesis testing. The new markers of inter-individual genomic and early phenotype variation developed as a result of this project will provide new reagents for individual diagnosis and therapies.

A multi-disciplinary, multi-institutional GE^{3}LS team will address: (i) conceptions of health, disease, illness, normalcy & disability; (ii) socioeconomics of monogenic health services; (iii) informed choice to participate in monogenic disease research; (iv) professional & educational issues in the conduct of monogenic disease research.

Understanding the structure and organization of chromosomes, genes, transcripts and their corresponding variants is the first step towards systematic analysis of the normal function of genes and their regulation in an organism. Furthermore, annotation of the full range of human genomic variation provides a starting point for understanding inter-individual differences underlying various phenotypic conditions, including disease states. Canada's long-standing track record in genetic and genomic discovery, coupled with a comprehensive health care network, creates an unrivalled opportunity to make an international impact in the discovery of all types of human genetic variations and mechanisms of disease. Ultimately, our genomic discoveries will provide both fundamental and applied information contributing to the diagnosis and treatment of illness, spearheading improvements in health care for Canadians and for communities world-wide.

3. Objectives of the Program Grant

Cellular Programs and Responses in Atherosclerosis: Linking Genotype to Phenotype

Our Central Theme is: *A key to understanding atherosclerosis is the elucidation of genetic determinants and ascertaining the impact of these determinants on metabolic outcomes and vascular cell responses - the phenomics of atherosclerosis.*

Objectives and Achievements from the Currently Funded HSFO Program

This application is the renewal of an HSFO Program Grant that has been tremendously successful. Our group established 4 important lines of investigation to examine cell responses in atherosclerosis, which has effectively linked our 5 laboratories to a common objective. Our discoveries of gene expression profiles directed us towards novel genes, pathways, and mechanisms of gene regulation that underlie vascular cell responses and function. Through strategic admixture of our complimentary research technologies, approaches and skill sets, the productivity emanating from the 4 objectives has been excellent. This degree of success would not have been possible without the HSFO Program Grant.

Our objectives for 2001-2005 were:

1. To define differential gene expression profiles in smooth muscle cells (SMCs) and macrophages.
2. To determine how gene mutations and single nucleotide polymorphisms (SNPs) associated with dyslipidemia, insulin resistance and the metabolic syndrome influence vascular cells.
3. To elucidate inflammatory cascades in cardiovascular disease.
4. To understand the relationship between alterations in G protein-coupled receptor (GPCR) signaling and vascular cell responses.

Progress:

Over the past 4 years, our HSFO group peer-reviewed publication record has been excellent and each manuscript has benefited from the programmatic approach we have adopted. Of significance, 25 of these manuscripts constituted formal collaborations between group PIs, as documented by shared authorships. Here we provide selected examples of our productivity in which major discoveries were made;, these advances would not have occurred without this integrative program.

Discovery of novel pathways driving diverse SMC phenotypes. We were the first, and to date only, group to successfully clone non-transformed SMCs from the media of the normal human adult artery. In doing so, we discovered distinct SMC subtypes. We have capitalized on this discovery, and the unique differentiation capacity of one SMC subtype, by undertaking microarray analysis and functional studies of putative novel SMC regulators. Of the many noteworthy findings, we discovered that lipoprotein lipase was differentially expressed and that this enzyme imparted to a subpopulation of SMCs a strong predisposition to accumulate lipids and differentiate into foam cells. We also discovered that Pre B-cell colony-enhancing factor regulates histone deacetylation and drives SMCs to a quiescent state, which attenuates the aging process. These findings opened an entirely new paradigm for foam cell formation and a novel cascade regulating SMC function and viability, with important consequences for plaque stabilization and potential therapy. These discoveries arose from collaborations between Drs. Huff, Pickering, and Hegele and have been published in *Circ Res* (2001), *ATVB* (2004), and *Circ Res* (2005).

Influence of gene mutations and variants, associated with dyslipidemia and the metabolic syndrome on vascular cells: We successfully applied genomic technologies to be the first in the world to discover the genetic basis of human monogenic forms of atherosclerosis, including familial partial lipodystrophy (FPLD) types 2 and 3, due respectively to mutations in *LMNA*, encoding nuclear lamin A/C, and *PPARG*, encoding peroxisomal proliferator-activated receptor gamma. These discoveries quickly lead to identification of novel pre-symptomatic metabolic phenotypes of insulin resistance that are predictors of future disease risk, together with novel vascular phenotypes ascertained through *in vivo* carotid ultrasound in affected subjects with familial partial lipodystrophy (FPLD). In other studies, we discovered and characterized at the transcriptional level a functional promoter variant in *PCK1*, encoding phosphoenol-pyruvate carboxykinase-1, the key enzyme in gluconeogenesis, and then

substantial strides towards elucidating genomic determinants of atherosclerosis, of its risk factors and of vascular cell behaviour. Our research direction is both to expand the repertoire of genetic/genomic players and then to link these with structural and functional phenotypes in humans, animal models and vascular cells. Our key experimental paradigms are: 1) gene discovery in model systems, including ethnic communities, vulnerable families, animals, organs, tissues and cells; 2) characterization of genotype-phenotype relationships, including intervention studies, in human monogenic disease states and in other model systems; 3) microanalyses of vulnerable plaque heterogeneity, linking morphology, cellular and molecular pathways, and the transcriptome.

One important context in which to examine these paradigms is the MetS, a phenotype found in 25% of Canadians that is a potent risk factor for atherosclerosis. Our strategy to understand MetS is to study rare human monogenic forms, such as FPLD and human genetic variants causing hyperlipidemia, hypertension, obesity, diabetes and vascular traits. Genes discovered using this paradigm provide the springboard for the application of our full spectrum of scientific expertise and core technologies.

Proposed research: In research supported by GIAs 1, 2, 3 and 5, Drs. Hegele, Pickering and Huff will advance understanding of atherosclerosis-related traits by: a) identifying mutations in new genes; b) extending the mutational spectrum in monogenic forms of disease in which the gene is already known; c) using functional assays to define disease mechanisms that are caused by specific human gene variants; and d) extending metabolic and vascular phenotypes in molecularly-characterized patients and disease families. Phenotyping methods include extensive analyses of a wide range of biochemical markers and metabolic indices together with the use of novel imaging modalities (such as 3D-ultrasound and MRI) to expedite whole patient phenotyping with a focus on vascular structure and fat distribution profiles. Additional phenotypes include *in vivo* plaque morphology, plaque compartmentalization using laser capture microscopy and expression analysis of aging genes.

Determinants of human atherosclerosis. We will apply our genomic analysis platforms, including expression arrays, 500K SNP DNA arrays for both large-scale copy number variation and association analyses, together with state-of-the-art bioinformatics methods, in order to identify new gene clusters, genes and/or variants that are associated with metabolic and/or vascular phenotypes. Gene variants so identified can be examined for association with human phenotypes in independent replication samples. Validated novel genes and variants associated with metabolic or vascular phenotypes at the population level can then be extensively studied using biochemical, cellular and molecular biological approaches in order to expand our understanding of new pathways. Conversely, candidate genes arising from unbiased experiments in model systems can be studied for association with MetS, dyslipidemia and accelerated atherosclerosis in families and populations. Examples of functionally characterized genes in which variants were or will be identified and studied for phenotype associations in various populations include *PBEF*, *WTAP*, *HSP47*, *fMLP*, *NPC1L1*, *AC6*, and *GPR30*. We will extend novel disease families and compare phenotypes between different molecular forms of the same gene product, which will allow us to define variability in the phenotype. We will combine this information with strategic use of functional experiments in animal and/or cell models. These approaches used together will elucidate novel pathways in pathogenesis of vascular disease. As we have repeatedly demonstrated, human disease mutations can serve as probes for new pathways or mechanisms.

Determinants of insulin-related phenotypes in atherosclerosis. Insulin resistance is associated with a specific pattern of hepatic gene expression that is associated with overproduction of atherogenic lipoproteins, partly as a consequence of the failure of insulin to activate specific intracellular signaling pathways. We discovered that, like insulin, naturally occurring molecules called flavonoids reverse this pattern of gene expression. Importantly, these flavonoids activate insulin signaling pathways independent of the insulin receptor, potentially via a novel GPCR. Experiments in cultured human hepatocytes and in diet-induced insulin-resistant LDL-receptor knockout mice are proposed to implicate specific components of the cell signaling pathways and to determine whether flavonoids attenuate insulin resistance and atherogenesis.

Determinants of cellular aging in atherosclerosis. Genotype-phenotype relationships will also be studied in the milieu of cellular aging and atherosclerotic plaque stability. Atherosclerotic plaques are exposed to stresses that can accelerate cellular aging (by oxidation and replication) which could, in

enhanced oxysterol synthesis on atherogenesis and plaque composition will be assessed *in vivo* in LDL-receptor knockout mice. The proposed link to activation of LXR-mediated gene expression will be tested in detail using laser capture microscopy to dissect subpopulations of arterial cells.

Objective 3. Defining novel signaling pathways that dynamically regulate vascular cell function.

Introduction: G protein-coupled receptors (GPCRs) play a central role in regulating vascular SMC responses to circulating and locally released hormones. The regulation of vascular smooth muscle tone represents a balance between endothelial and vascular smooth muscle receptor-mediated mechanisms of regulation, the activation of GPCRs linked to vasodilation and vasoconstriction and GPCR-regulated signaling linked to proliferation. Moreover, the activity of the GPCRs regulating these pathways is modulated by intracellular mechanisms that define receptor G protein-coupling, desensitization and resensitization. We discovered that the steroid hormone aldosterone mediates acute effects on vascular smooth muscle tone by mediating contractile responses potentially through the activation of the orphan GPCR GPR-30. In addition, the activity of different GPCRs linked to vasodilation and vasoconstriction of vascular SMCs can be differentially regulated based on differences in: 1) their intracellular trafficking patterns between various endosomal compartments; 2) their association with different Rab GTPases and 3) their propensity to either resensitize or remain desensitized.

Proposed Research: Research supported by GIAs 6, 7 and 8, Drs. Feldman, Ferguson and Pickering will examine the impact of novel components of GPCR signaling on vascular SMC function.

Determinants of vascular tone. We will exploit our recent discovery that aldosterone acutely regulates vascular tone through GPCR signaling. We will test the hypothesis that aldosterone mediates its acute effects in SMCs via the activation of the orphan GPCR, GPR-30. The biochemical consequences in human SMCs challenged acutely with aldosterone will be examined following the targeted knockdown by short hairpin RNA or overexpression of either GPR-30 or the mineralocorticoid receptor, using viral vectors. Functional consequences will be evaluated in single cells by dynamic time-lapse imaging. The concept of aldosterone-mediated acute activation of GPCR signaling will be extended to endothelial cells and intact vessels in organ culture using our well developed model systems. The role of this aldosterone-induced signaling in the pathogenesis of hypertension will be elucidated *in vivo* using genetic (SHR) and acquired (Dahl salt-sensitive) rat models of hypertension and ultimately in humans with hypertension using gluteal biopsies and perfusion myography.

Cellular determinants of GPCR signaling in the vasculature. We will capitalize on our discovery that specific RabGTPases are critical for GPCR recycling. This has led us to hypothesize that Rab GTPases critially regulate human SMC contractile function by orchestrating the endocytosis, trafficking, recycling and resensitization of the β_2 adrenergic receptor and angiotensin-1 receptor. We will assess whether the resensitization of endogenous β_2AR and AT_{1A}R in adult human arterial SMCs is altered by the adenoviral-induced expression of dominant-negative or constitutively active GFP-tagged Rab5, Rab4 and Rab11 proteins. Using confocal microscopy and biochemical determinations, we will determine whether these Rab GTPases regulate the endocytosis and trafficking of GPCRs to endosomes (Rab5) as well as the recycling of receptors back to the cell surface via rapid (Rab4) and slow (Rab11) recycling endosomes. Alterations in SMC-mediated vascular responsiveness will be assessed in organ cultures from rat aortic and mesenteric arteries transfected with adenoviral constructs encoding dominant negative and constitutively active Rab GTPases. The long-term goal of these experiments will be to develop mice in which Rab GTPases are over-expressed or knocked out, in a vascular smooth muscle-specific manner, and assess the impact on hypertension and predisposition to atherosclerosis.

Summary

This program will create significant synergies resulting in the establishment of coherent lines of investigation that link our laboratories to a central theme -- the phenomics of atherosclerosis. Our proposed studies in humans, *ex vivo* tissues, animal models and cells will capitalize on state-of-the-art technologies to elucidate genetic determinants of atherosclerosis and their impact on metabolism and vascular function. Our results will allow us to identify novel pathways involved in the pathogenesis of this complex disease and provide an enhanced and improved rationale for the diagnosis and treatment of atherosclerosis.

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Description

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SECTION I
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Frozen cultures may contain 5 to 10% Dimethyl sulfoxide (DMSO)

SECTION II
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SECTION III
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This cell line is not known to harbor an agent known to cause disease in healthy adult humans. This cell line has **NOT** been screened for Hepatitis B, human immunodeficiency viruses or other adventitious agents. Handle as a potentially biohazardous material under at least Biosafety Level 1 containment.

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SECTION IV
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Not applicable

Cell Biology

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Related Cell Culture Products

Applications:	transfection host (technology from amaxa Roche FuGENE® Transfection Reagents)
Receptors:	insulin, expressed
Reverse Transcript:	negative
Age:	embryo
Comments:	L1 is a continuous substrain of 3T3 (Swiss albino) developed through clonal isolation. The cells undergo a pre-adipose to adipose like conversion as they progress from a rapidly dividing to a confluent and contact inhibited state. A high serum content in the medium enhances fat accumulation [PubMed ID: 4426090]. Tested and found negative for ectromelia virus (mousepox). This line is also designated as ATCC CCL-92.1.ATCC ® CL-173 was deposited in 1974 without passage number information from the depositor. At the time of submission, ATCC prepared approximately 30 vials of seed stock at about 4 passages beyond the original depositor material (passage number: unknown +4).
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** The serum used is important in culturing this line. Calf

serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

Protocol: Never allow culture to become completely confluent.

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels. The recommended inoculum is 2 to 3 X 10(3) cells/sq. cm. Subculture before cultures become 70 to 80% confluent or when cells reach 5 to 6 X10 (4) viable cells/sq. cm.
6. Incubate cultures at 37C.

Interval: Every three days
Medium Renewal: 2 to 3 times per week

Preservation:
Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Doubling Time: 14 hrs

Related Products:
formerly distributed as:ATCC CCL-92.1
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Cell culture tested DMSO:ATCC 4-X
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

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Cell Biology

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Related Cell Culture Products

Applications:
transfection host (technology from amaxa
Roche FuGENE® Transfection Reagents)

Virus Susceptibility: Murine leukemia virus

Age: embryo

Comments:
The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies [PubMed ID: 222457].
Tested and found negative for ectromelia virus (mousepox).

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

Subculturing:
Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal.

4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37C.

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DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less.
Subcultivation Ratio: Inoculate 3 to 5 X 10(3) cells/cm2
Medium Renewal: Twice per week

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

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Cell Biology

ATCC® Number:	**CRL-1651™** Order this Item	Price:	**$264.00**
Designations:	COS-7	Depositors:	Y Gluzman
Biosafety Level:	2 [Cells Contain SV-40 viral DNA sequences]	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent fibroblast
Organism:	*Cercopithecus aethiops*	Morphology:	
Source:	**Organ:** kidney **Cell Type:** SV40 transformed		
Cellular Products:	T antigen		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

Related Cell Culture Products

Applications:	transfection host(technology from amaxa Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	SV40 (lytic growth); SV40 tsA209 at 40C; SV40 mutants with deletions in the early region
Comments:	This is an African green monkey kidney fibroblast-like cell line suitable for transfection by vectors requiring expression of SV40 T antigen. This line contains T antigen, retains complete permissiveness for lytic growth of SV40, supports the replication of ts A209 virus at 40C, and supports the replication of pure populations of SV40 mutants with deletions in the early region.The line was derived from the CV-1 cell line (ATCC ® CCL-70?) by transformation with an origin defective mutant of SV40 which codes for wild type T antigen.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach

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Product Description

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

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Cell Biology

ATCC® Number:	**CRL-1573™** Order this Item	**Price:**	**$256.00**
Designations:	293 [HEK-293]	**Depositors:**	FL Graham
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial PHOTO
Source:	**Organ:** kidney **Cell Type:** transformed with adenovirus 5 DNA		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
	Related Cell Culture Products		
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.		
Applications:	efficacy testing [92587] transfection host (technology from amaxa Roche FuGENE® Transfection Reagents) viruscide testing [92579]		
Receptors:	vitronectin, expressed		
Tumorigenic:	YES		
DNA Profile (STR):	Amelogenin: X CSF1PO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19		
Cytogenetic Analysis:	This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t (8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.		
Age:	fetus		
Comments:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768] The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]		

The Ad5 insert was cloned and sequenced, and it was determined that a colinear seqment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached.The cells will re-attach to the flask over a period of several days in culture at 37C.

Subculturing:

Protocol:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.An inoculum of 2 X 10 (3) to 6 X 10 (3) viable cells/cm2 is recommended.
6. Incubate cultures at 37°C.6. Subculture when cell concentration is between 6 and 7 X 10(4) cells/cm2.

Subcultivation Ratio: 1:10 to 1:20 weekly.
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003
derivative: ATCC CRL-10852
derivative: ATCC CRL-12006
derivative: ATCC CRL-12007
derivative: ATCC CRL-12013
derivative: ATCC CRL-12479
derivative: ATCC CRL-2029
derivative: ATCC CRL-2368
purified DNA:ATCC CRL-1573D

References:

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Cell Biology

ATCC® Number:	**CRL-11997™** Order this Item	Price:	**$417.00**
Designations:	HEP G2/2.2.1	Depositors:	Northeastern Ohio University College of Medicine
Biosafety Level:	1	Shipped:	frozen
Medium & Serum:	See Propagation	Growth Properties:	adherent
Organism:	*Homo sapiens* (human)	Morphology:	epithelial
Source:	**Organ:** liver **Disease:** hepatocellular carcinoma		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

This material is cited in a U.S. and/or other Patent or Patent Application, and may not be used to infringe on the patent claims. ATCC is required to inform the Patent Depositor of the party to which the material was furnished.

Related Cell Culture Products

DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 9,13 D16S539: 12,13 D5S818: 11,12 D7S820: 10 THO1: 9 TPOX: 8,9 vWA: 17
Age:	15 years
Gender:	male
Ethnicity:	Caucasian
Comments:	Cell line was derived from the hepatocellular carcinoma cell line, HepG2 (ATCC HB-8065). The parental cells were stably transfected at passage 48 with a human cholesterol 7 alpha-hydroxylase (CYP7) minigene/Luciferase construct.
Propagation:	**ATCC complete growth medium:** A 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F12 medium with 2.5 mM L-glutamine, 15 mM HEPES, 0.5 mM sodium pyruvate and 1200 mg/L sodium bicarbonate and supplemented with 0.4 mg/ml G418, 90%; fetal bovine serum, 10% **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C **Growth Conditions:** These cells are slow to attach after subculture. Allow 4 to 5 days for reattachment.

Protocol:

Subculturing:

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

Subcultivation Ratio: A subcultivation ratio of 1:4 to 1:6 is recommended
Medium Renewal: Every 2 to 3 days

Preservation:

Freeze medium: Complete growth medium supplemented with 5% (v/v) DMSO
Storage temperature: liquid nitrogen vapor phase

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2006
parental cell line:ATCC HB-8065
recommended serum:ATCC 30-2020

References:

40153: Chiang JY, Stroup D. Assay for agents that affect cholesterol 7alpha-hydroxylase expression and a characterization of its regulatory elements. US Patent 5,821,057 dated Oct 13 1998

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Minimum Safety Guidelines
Recommended for Working with Human and Animal Cell Cultures

BIOSAFETY LEVEL

It is recommended that all cell cultures distributed by Coriell Cell Repositories be handled according to appropriate Biosafety Level guidelines established by U.S. Department of Health and Human Service, Centers for Disease Control and Prevention and the National Institutes of Health. The HHS publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition, contains details of practices and schedules of pathogens according to Biosafety Level.

Coriell Cell Repositories recommends that (unless otherwise indicated) all CCR human and animal cell lines be handled at **BioSafety Level 2**.

SUPERVISION

Administrative Responsibilities
Responsibility of Management: Management should establish a biohazards committee to institute and enforce a health and safety policy which includes a specific safety program for work involving human cell lines. The program should meet applicable federal, state, and local regulations and include safety training, maintenance of accident records, and provisions for emergency treatment.

Responsibility of the Principal Investigator: The principal investigator is responsible for the preparation of safety protocols for the research program under his or her direction. The protocols should include appropriate procedures for use, storage, decontamination, disposal, and emergency treatment. The protocols should be approved by the biohazard committee and discussed with the research staff before starting the research program.

Medical Surveillance and Screening:
Physical Examinations: Appropriate pre-employment and periodic medical examinations are desirable for persons working with human cell lines.

Work Restrictions: Persons having reduced immunologic competency should be restricted from working with these human cell lines.

Serum Collection and Banking: Serum should be collected at the time of employment to establish a baseline reference. Serum should be collected immediately after accidental injection or ingestion and at an appropriate interval thereafter. For additional information, see CDC publication *Biosafety in Microbiological and Biomedical Laboratories* (BMBL) 5th Edition.

Laboratory Access
Access to the cell culture area should be restricted to persons working directly with the cell lines, or by specific authorization by the principal investigator or director of the laboratory.

PERSONNEL PRACTICES

Pipetting: Mechanical pipetting aids rather than mouth pipetting should be used for all pipetting procedures.

Eating, Drinking, and Smoking: Eating, drinking, and smoking should not be allowed in the same areas where cell lines are under study.

Protective Clothing: It is recognized that the criteria for protective clothing may vary according to the physical situation of the laboratory and the agents handled. Ideally, adequate protective clothing such as a fully fastened laboratory coat should be worn. This clothing should not be worn outside the work area once the work area has been entered.

PHYSICAL CONTROL PRACTICES

Recommended for all cell lines, but required for long-term lymphoid lines and their derivatives.

Ventilated Safety Cabinets or Hoods: Ventilated safety cabinets and hoods and other safety apparatus should be employed and should be tested at least annually to certify correct containment and operation. A list of specifications for satisfactory hoods and instructional materials may be obtained from the Office of Biohazards, NCI.

Housekeeping: Appropriate housekeeping procedures which suppress the formation of aerosols should be used. Work surfaces should be wiped down with an appropriate disinfectant before and after work with each cell culture and at the end of the workday.

Decontamination and Disposal: Contaminated glassware and similar materials should be appropriately decontaminated or stored for decontamination before removal from the work area for recycling or disposal. Liquid wastes should be decontaminated either chemically or by heat before being discharged to the community sanitary sewer system.

Protection of Vacuum Lines: Vacuum services, if used, should be protected with disposable absolute air filters and liquid traps. The effluent should be collected in liquid traps containing concentrated disinfectant.

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Catalog ID: **GM05659** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Chest
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 1 YR
Sex Male
Race Caucasian
Relation to Proband proband
Clinically Affected No
Confirmation Clinical summary/Case history
Remarks Skin biopsy (chest); 46,XY

Catalog ID GM05659
Product Cell Culture
Pricing Commercial Pricing: $85.00
Academic and not-for-profit pricing: $85.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
PDL at Freeze 16
Passage Frozen 8

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

Phenotypic Data

Remark Skin biopsy (chest); 46,XY

Publications

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External Links

dbSNP dbSNP ID: 20062
GEO GEO Accession No: GSM217867

Images

View karyotype
karyotype

Protocols

PDL at Freeze 16
Passage Frozen 8
Split Ratio 1:4
Temperature 37 C
Percent CO2 5%
Percent O2 AMBIENT
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

Catalog ID: **GM08398** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Inguinal area
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 8 YR
Sex Male
Race Caucasian
Relation to Proband proband
Clinically Affected No
Confirmation Clinical summary/Case history
ISCN 46,XY
Remarks Skin biopsy (inguinal)

Catalog ID GM08398
Product Cell Culture
Pricing Commercial Pricing: $85.00
Academic and not-for-profit pricing: $85.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
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

Phenotypic Data

Remark	Skin biopsy (inguinal)

Publications

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External Links

dbSNP dbSNP ID: 21062
GEO GEO Accession No: GSM88295
GEO Accession No: GSM88296
GEO Accession No: GSM88297
GEO Accession No: GSM88313
GEO Accession No: GSM88314
GEO Accession No: GSM88315

Images

View karyotype

Protocols

Passage Frozen 3
Split Ratio 1:4
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: **GM03348** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIGMS Human Genetic Cell Repository
Subcollection Apparently Healthy Collection
Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Biopsy Source Inguinal area
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 10 YR
Sex Male
Race Caucasian
Relation to Proband proband
Clinically Affected No
Confirmation Karyotypic analysis and Case history
ISCN 46,XY
Remarks Skin biopsy (inguinal); 46,XY

Catalog ID GM03348
Product Cell Culture
Pricing Commercial Pricing: $85.00
Academic and not-for-profit pricing: $85.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description APPARENTLY HEALTHY NON-FETAL TISSUE
Passage Frozen 7

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

Phenotypic Data

Remark Skin biopsy (inguinal); 46,XY

Publications

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The FASEB journal : official publication of the Federation of American Societies for Experimental Biology17:1706-8 2003
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PubMed ID: 6270157

External Links

dbSNP dbSNP ID: 18209

Images

View karyotype
karyotype

Protocols

Passage Frozen 7
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: **AG03513** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection Inherited Disorders
Sample Description HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS
Biopsy Source Arm
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 13 YR
Sex Male
Race Caucasian
Ethnicity MEXICAN
Family 172
Family Member 1
Relation to Proband proband
Clinically Affected Yes
Confirmation Clinical summary/Case history
ISCN 46,XY
Remarks Donor has classical features of progeria, but no signs of heart disease. Parents and sibs are clinically unaffected. The biopsy was taken antemortem on 5/15/79 from skin on the medial forearm. The culture was initiated using explants of minced skin tissue. The cell morphology is fibroblast-like. The karyotype is 46,XY; normal diploid male. An increased thermolabile component of triosephosphate isomerase has been reported for this culture. A lymphoblast culture from same donor is AG03506.

Catalog ID AG03513
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS
PDL at Freeze 15
Passage Frozen 8

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

Phenotypic Data

Remark Donor has classical features of progeria, but no signs of heart disease. Parents and sibs are clinically unaffected. The biopsy was taken antemortem on 5/15/79 from skin on the medial forearm. The culture was initiated using explants of minced skin tissue. The cell morphology is fibroblast-like. The karyotype is 46,XY; normal diploid male. An increased thermolabile component of triosephosphate isomerase has been reported for this culture. A lymphoblast culture from same donor is AG03506.

Publications

Glynn MW, Glover TW, Incomplete processing of mutant lamin A in Hutchinson-Gilford progeria leads to nuclear abnormalities, which are reversed by farnesyltransferase inhibition Human molecular genetics14:2959-69 2005
PubMed ID: 16126733

Huang S, Chen L, Libina N, Janes J, Martin GM, Campisi J, Oshima J, Correction of cellular phenotypes of Hutchinson-Gilford Progeria cells by RNA interference Human genetics118:444-50 2005
PubMed ID: 16208517

Reddel CJ, Weiss AS, Lamin A expression levels are unperturbed at the normal and mutant alleles but display partial splice site selection in Hutchinson-Gilford progeria syndrome Journal of medical genetics41:715-7 2004
PubMed ID: 15342704

Giro M, Davidson JM, Familial co-segregation of the elastin phenotype in skin fibroblasts from Hutchinson-Gilford progeria. Mech Ageing Dev70:163-36 1993
PubMed ID: 8246632

Winkles JA, O'Connor ML, Friesel R, Altered regulation of platelet-derived growth factor A-chain and c-fos gene expression in senescent progeria fibroblasts. J Cell Physiol144:313-25 1990
PubMed ID: 2166059

Sephel GC, Sturrock A, Giro MG, Davidson JM, Increased elastin production by progeria skin fibroblasts is controlled by the steady-state levels of elastin mRNA. J Invest Dermatol90:643-7 1988
PubMed ID: 3361140

Nakamura KD, Turturro A, Hart RW, Elevated c-myc expression in progeria fibroblasts. Biochem Biophys Res Commun155:996-1000 1988
PubMed ID: 3421979

Oliver CN, Ahn BW, Moerman EJ, Goldstein S, Stadtman ER, Age-related changes in oxidized proteins. J Biol Chem262:5488-91 1987
PubMed ID: 3571220

Mokrasch LC, Cell membrane transport enzymes in cultured dermal fibroblasts from progeroid donors: a comparison to other human fibroblasts. Gerontology32:202-6 1986
PubMed ID: 3770489

Chapman ML, Zaun MR, Gracy RW, Changes in NAD levels in human lymphocytes and fibroblasts during aging and in premature aging syndromes. Mech Ageing Dev21:157-67 1983
PubMed ID: 6223188

Tollefsbol TO, Zaun MR, Gracy RW, Increased lability of triosephosphate isomerase in progeria and Werner's syndrome fibroblasts. Mech Ageing Dev20:93-101 1982
PubMed ID: 7176709

External Links

dbSNP	dbSNP ID: 14675
OMIM	176670 HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS
Omim Description	HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS PROGERIA

Images

Data are not available

Protocols

PDL at Freeze	15
Passage Frozen	8
Split Ratio	1:4
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: **AG04456** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection GRC
Sample Description GERONTOLOGY RESEARCH CENTER (GRC) CELL CULTURE COLLECTION BALTIMORE LONGITUDINAL STUDY ON AGING (BLSA)
Biopsy Source Arm
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 49 YR
Sex Male
Race Caucasian
Family 878
Family Member 1
Relation to Proband proband
Clinically Affected No
Confirmation Clinical summary/Case history
ISCN 46,XY/46,XY,t(1;6)(1pter>1q25::6q13> 6qter;6pter>6q13::1q25>1qter)
Remarks The biopsy site is the mesial aspect of the mid-upper left arm, taken antemortem. The primary culture was initiated from explants of a 2 mm-punch biopsy. The cell morphology is fibroblast-like. The culture is a mosaic with karyotype: 46,XY/46,XY,t(1;6) (1pter>1q25::6q13>6qter;6pter>6q13::1q25> 1qter); balanced; 44%/56%. A culture initiated from a biopsy taken 19 years later from this same donor is AG14251. Donor is the son of AG04459.

Catalog ID AG04456
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description GERONTOLOGY RESEARCH CENTER (GRC) CELL CULTURE COLLECTION BALTIMORE LONGITUDINAL STUDY ON AGING (BLSA)
PDL at Senescence 41
PDL at Freeze 11

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

Phenotypic Data

Remark The biopsy site is the mesial aspect of the mid-upper left arm, taken antemortem. The primary culture was initiated from explants of a 2 mm-punch biopsy. The cell morphology is fibroblast-like. The culture is a mosaic with karyotype: 46,XY/46,XY,t(1;6)(1pter>1q25::6q13>6qter;6pter>6q13::1q25> 1qter); balanced; 44%/56%. A culture initiated from a biopsy taken 19 years later from this same donor is AG14251. Donor is the son of AG04459.

Publications

Kriete A, Mayo KL, Yalamanchili N, Beggs W, Bender P, Kari C, Rodeck U, Cell autonomous expression of inflammatory genes in biologically aged fibroblasts associated with elevated NF-kappaB activity Immunity & ageing : I & A5:5 2008
PubMed ID: 18631391

Schneider EL, Monticone R, Smith J, Braunschweiger K, Roberts T, Skin fibroblast cultures derived from members of the Baltimore longitudinal study: a new resource for studies of cellular aging. Cytogenet Cell Genet31:40-6 1981
PubMed ID: 7307581

External Links

dbSNP dbSNP ID: 20738

Images

Data are not available

Protocols

PDL at Senescence	41
PDL at Freeze	11
Split Ratio	1:2
Temperature	37 C
Medium	Eagle's MEM with Hank's BSS, with 26mM Hepes; ambient CO2 with 2mM L-glutamine
Serum	15% fetal bovine serum Not inactivated
Substrate	None specified
Subcultivation Method	trypsin-EDTA

April 22/09

Catalog ID: **AG06234** Product (Source): CELL CULTURE · ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection GRC
Sample Description GERONTOLOGY RESEARCH CENTER (GRC) CELL CULTURE COLLECTION
BALTIMORE LONGITUDINAL STUDY ON AGING (BLSA)
Biopsy Source Arm
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 17 YR
Sex Male
Race Caucasian
Family 939
Family Member 2
Relation to Proband son
Clinically Affected No
Confirmation Clinical summary/Case history
Remarks The biopsy site is the mesial aspect of the mid-upper left arm, taken antemortem. The primary culture was initiated from explants of a 2 mm-punch biopsy. The cell morphology is fibroblast-like and the karyotype is 46,XY; diploid male. The donor is the son of AG06235 and AG08692. A culture initiated from a biopsy taken 13 years later from this same donor is AG13153.

Catalog ID AG06234
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description GERONTOLOGY RESEARCH CENTER (GRC) CELL CULTURE COLLECTION
BALTIMORE LONGITUDINAL STUDY ON AGING (BLSA)
PDL at Freeze 17.74

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

Phenotypic Data

Remark The biopsy site is the mesial aspect of the mid-upper left arm, taken antemortem. The primary culture was initiated from explants of a 2 mm-punch biopsy. The cell morphology is fibroblast-like and the karyotype is 46,XY; diploid male. The donor is the son of AG06235 and AG08692. A culture initiated from a biopsy taken 13 years later from this same donor is AG13153.

Publications

Miyoshi N, Oubrahim H, Chock PB, Stadtman ER, Age-dependent cell death and the role of ATP in hydrogen peroxide-induced apoptosis and necrosis Proceedings of the National Academy of Sciences of the United States of America103:1727-31 2006
PubMed ID: 16443681

Smilenov LB, Dhar S, Pandita TK, Altered telomere nuclear matrix interactions and nucleosomal periodicity in ataxia telangiectasia cells before and after ionizing radiation treatment. Mol Cell Biol19:6963-71 1999
PubMed ID: 10490633

Cristofalo VJ, Allen RG, Pignolo RJ, Martin BG, Beck JC, Relationship between donor age and the replicative lifespan of human cells in culture: a reevaluation. Proc Natl Acad Sci U S A95:10614-9 1998
PubMed ID: 9724752

Allen RG, Keogh BP, Gerhard GS, Pignolo R, Horton J, Cristofalo VJ, Expression and regulation of superoxide dismutase activity in human skin fibroblasts from donors of different ages. J Cell Physiol165:576-87 1995

Amn 22/09

Catalog ID: **AG06297** Product (Source): CELL CULTURE ADD TO CART

Overview

Collection NIA Aging Cell Culture Repository
Subcollection Inherited Disorders
Sample Description HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS
LAMIN A/C; LMNA
Biopsy Source Thigh
Cell Type Fibroblast
Tissue Type Skin
Transformant Untransformed
Species Homo sapiens
Common Name Human
Age 8 YR
Sex Male
Race Caucasian
Relation to Proband proband
Clinically Affected Yes
Confirmation Clinical summary/Case history
Remarks The donor had classic features of progeria and died at age 14 years of congestive heart failure. Autopsy revealed severe coronary-vascular disease. The biopsy was taken ante-mortem on 1/11/72 from skin on the anterior thigh region. The culture was initiated using explants of minced skin tissue. The cell morphology is fibroblast-like. The karyotype is 46,XY; normal diploid male. Culture is frozen at PDL 33 and has a maximum lifespan of 40 PD. Donor subject has a de novo single base substitution, a C>T change at nucleotide 2036 (2036C>T), which results in a silent change at codon 608 [Gly608Gly (G608G)] in exon 11 of the Lamin A gene (LMNA). This substitution creates an exonic consensus splice donor sequence and results in activation of a cryptic splice site which in turn causes skipping of 150 bp of the LMNA mRNA leading to the deletion of 50 amino acids from the protein. This altered LMNA protein was detected on western blots [Eriksson et al., Nature 423:293 (2003)].

Catalog ID AG06297
Product Cell Culture
Pricing Commercial Pricing: $155.00
Academic and not-for-profit pricing: $85.00
NIA Grantees: $0.00
Forms Required for Ordering Assurance Form (Must have current form on file)
Order Form (Download if ordering by FAX)
Statement of Research Intent Form (Download if ordering by FAX)

Characterizations

Sample Description HUTCHINSON-GILFORD PROGERIA SYNDROME; HGPS
LAMIN A/C; LMNA
PDL at Freeze 39.08
Passage Frozen 18

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

Gene LMNA
Chromosomal Location 1q21.2
Allelic Variant 1 150330.0022; HUTCHINSON-GILFORD PROGERIA SYNDROME
Identified Mutation **GLY608GLY**; Description: In 18 of 20 patients with classic Hutchinson-Gilford progeria syndrome (176670), Eriksson et al. [Nature 423: 293 (2003)] found an identical de novo single-base substitution, a C-to-T change resulting in a silent gly-to-gly mutation at codon 608 (G608G) within exon 11 of the LMNA gene. This substitution created an exonic consensus splice donor sequence and resulted in activation of a cryptic splice site and deletion of 50 basepairs of prelamin A. This mutation was not identified in any of the 16 parents available for testing.

Phenotypic Data

Remark The donor had classic features of progeria and died at age 14 years of congestive heart failure. Autopsy revealed severe coronary-vascular disease. The biopsy was taken ante-mortem on 1/11/72 from skin on the anterior thigh region. The culture was initiated using explants of minced skin tissue. The cell morphology is fibroblast-like. The karyotype is 46,XY; normal diploid male. Culture is frozen at PDL 33 and has a maximum lifespan of 40 PD. Donor subject has a de novo single base substitution, a C>T change at nucleotide 2036 (2036C>T), which results in a silent change at codon 608 [Gly608Gly (G608G)] in exon 11 of the Lamin A gene (LMNA). This substitution creates an exonic consensus splice donor sequence and results in activation of a cryptic splice site which in turn causes skipping of 150 bp of the LMNA mRNA leading to the deletion of 50 amino acids from the protein. This altered LMNA protein was detected on western blots [Eriksson et al., Nature 423:293 (2003)].

Publications

Matthew Ban

From: Tony Rupar [Tony.Rupar@LHSC.ON.CA]
Sent: Monday, March 30, 2009 11:05 AM
To: Roger.Dewar@ontario.ca; mban@robarts.ca; Piya Lahiry
Cc: Rob Hegele; Tony Rupar
Subject: Re: MSDS for primary cell lines??

Hi Piya;

We receive these cell lines for diagnostic purposes in our diagnostic laboratory. Similarly we receive blood, urine, CSF, and other tissues for diagnostic purposes. Licensing, accreditation, standards etc for diagnostic laboratories are governed by Ontario Laboratory Association which is a joint responsibility of the OMA and MOHLTC. Under these rules we operate (and are inspected) under biohazard level 2 conditions but are not required to have MSDS's.

Hopefully this is helpful.

tony

>>> Piya Lahiry <plahiry2@uwo.ca> 2009/03/30 10:00 AM >>>
Hi Roger,
this is Piya Lahiry, from Dr. Hegele's lab. I was hoping to ask you administrative question. We are currently filling up our Biohazardous Agents Registry form for the Hegele Lab. I was hoping you could tell me how you record the cell lines that you have from the Amish population. Do you have 'MSDS' for this cell lines or how do you get approval from UWO Health and Safety?

pCMV6-Entry (C-terminal Myc and DDK Tagged)

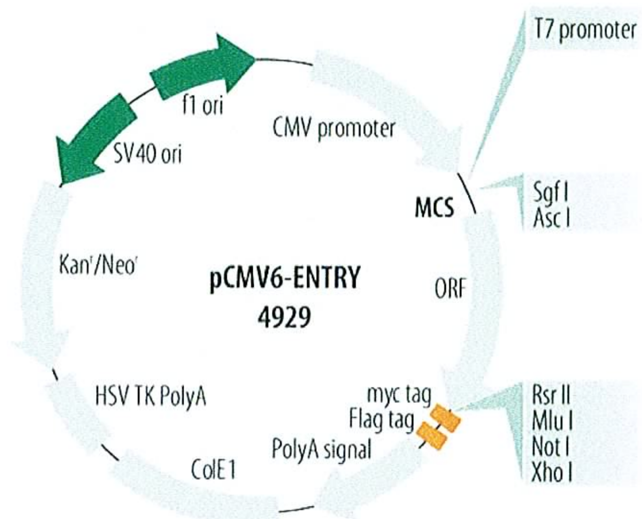
Specifications | Custom Cloning | Product Manual | FAQs

Catalog No.	Description	Price	Delivery
PS100001	pCMV6-Entry (C-terminal Myc and DDK Tagged)	$320	Immediate

Add to Shopping Cart

Features:

- ORFs cloned in this vector will be expressed in mammalian cellsas a tagged protein with the C-terminal Myc-DDK tags. (DDK is the same as FLAG® which is a registered trademark of Sigma Aldrich).
- Such clones are the best for detection and purification of the transgene using anti-Myc or anti-DDK antibodies.
- Serve as the entry vector in the PrecisionShuttle system to transfer the ORF sequence into any destination vectors for other tagging options or other expression platforms.



Schematic of the multiple cloning sites:

```
                                                                              Kozac
                                                                            Consensus
                 EcoR I         BamH I Kpn I    RBS                          Sgf I      Asc I    Bgl II
CTATAGGGCGGCCGGGAATTCGTCGACTGGATCCGGTACCGAGGAGATCTGCCGCCGCGATCGCCGGCGCGCCAGATCT

Hind III            Rsr II    Mlu I         Not I       Xho I              Myc.Tag
CAAGCTTAACTAGCTAGCGGACCG ACG CGT ACG CGG CCG CTC GAG CAG AAA CTC ATC TCA GAA GAG
                          T   R   T   R   P   L   E   Q   K   L   I   S   E   E

                         EcoR V                Flag.Tag                         Pme I    Fse I
GAT CTG GCA GCA AAT GAT ATC CTG GAT TAC AAG GAT GAC GAC GAT AAG GTT TAA ACGGCCGGCC
 D   L   A   A   N   D   I   L   D   Y   K   D   D   D   D   K   V  Stop
```

Download full vector sequence

OriGene cDNAs in recent publications

Selective regulation of long-form calcium-permeable AMPA receptors by an atypical TARP Nature Neuroscience 12, 277 - 285 (01 Mar 2009), doi: 10.1038/nn.2266 [CACNG7 (TARP7)]

Mitogen-Activated Protein Kinase Inhibition Induces Translocation of Bmf to Promote Apoptosis in Melanoma Cancer Res., Mar 2009; 69: 1985 - 1994. [BCL2, BCL2A1, MCL1]

PTEN Loss Promotes Mitochondrially Dependent Type II Fas-Induced Apoptosis via PEA-15, Mol. Cell. Biol., Mar 2009; 29: 1222 - 1234. [PEA15]

S-Glutathionylation Impairs Signal Transducer and Activator of Transcription 3 Activation and Signaling, Endocrinology, Mar 2009; 150: 1122 - 1131. [STAT3]

A conditional transposon-based insertional mutagenesis screen for genes associated with mouse hepatocellular carcinoma, Nature Biotechnology (22 Feb 2009), doi: 10.1038/nbt.1526 [UBE2H]

Cadmium Toxicity toward Autophagy through ROS-Activated GSK-3ß in Mesangial Cells, Toxicol. Sci., Mar 2009; 108: 124 - 131. [GSK3B]

View all citations on TrueClone

Certificate of Analysis

Product:	Human cDNA clone
Catalog No:	RC214230
Accession No:	NM_001486.2
Gene Name:	ORF Clone of Homo sapiens glucokinase (hexokinase 4) regulator (GCKR) as transfection-ready DNA
Quantity:	1
Shipment Reviewed By/On:	JAN 20 2009
Components:	1 2-D bar-coded Matrix vial containing ten (10) micrograms dried DNA plasmid (reconstitute with 100 ul water) 1 Vial of dried 5' primer (100 picomoles) 1 Vial of dried 3' primer (100 picomoles) 1 Application Guide
Vector:	pCMV6 Entry (Myc/DDK tagged)
Antibiotic Resistance:	Kanamycin (25 ug/mL)
Restriction Site:	SgfI-MluI
Quality Control:	This plasmid has been derived from single colony E. coli cultures and purified through ion-exchange chromatography. The eluant is normalized via UV spectroscopy, aliquoted into a Matrix vial then dried. Detailed sequence information for this clone is found on the OriGene website by searching the catalog number above.
Storage Conditions:	Store the dried plasmid at -20 degrees C. Store the suspended plasmid at -20 degrees C.
Stability:	The dried and the suspended DNA is stable for one year from date of shipping when stored at -20 degrees C.

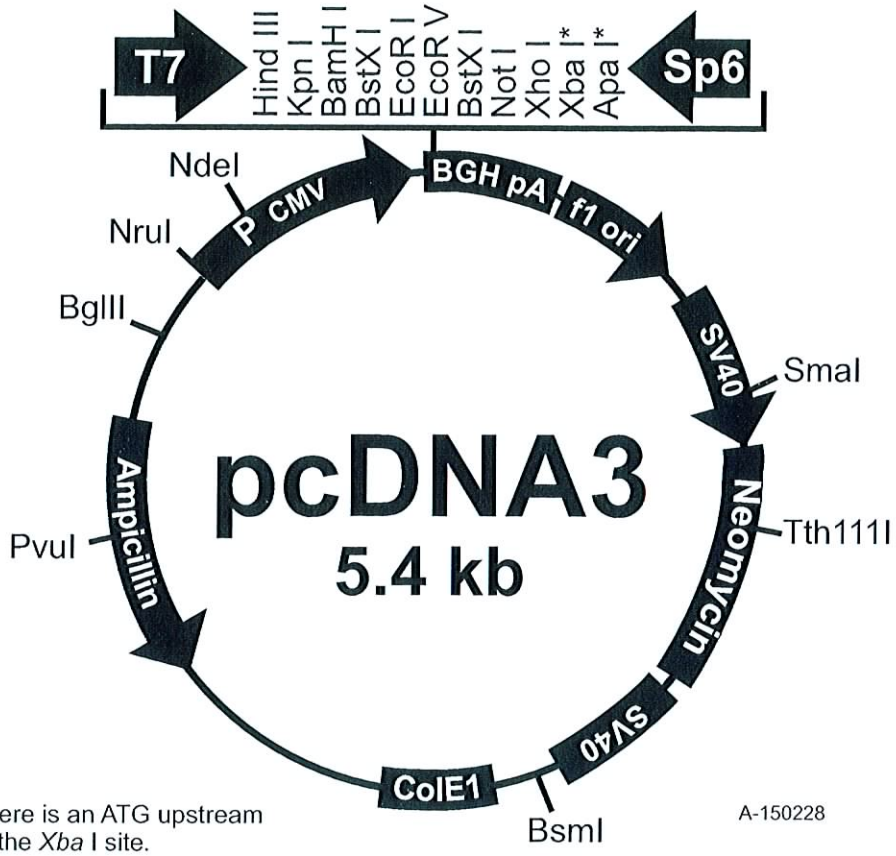
Product not intended for use in humans, for research purposes only.

ORIGENE
Your Gene Company

OriGene Technologies, Inc.
6 Taft Court, Suite 100
Rockville, MD 20850
phone: 888 267-4436
fax: 301 340-9254
http://www.origene.com

Comments for pcDNA3:
5446 nucleotides

CMV promoter: bases 209-863
T7 promoter: bases 864-882
Polylinker: bases 889-994
Sp6 promoter: bases 999-1016
BGH poly A: bases 1018-1249
SV40 promoter: bases 1790-2115
SV40 origin of replication: bases 1984-2069
Neomycin ORF: bases 2151-2945
SV40 poly A: bases 3000-3372
ColE1 origin: bases 3632-4305
Ampicillin ORF: bases 4450-5310



The sequence of pcDNA3 has been compiled from information in sequence databases, published sequences, and other sources. This vector has not yet been completely sequenced. If you suspect an error in the sequence, please contact Invitrogen's Technical Services Department.

invitrogen

Material Safety Data Sheet

Revision Date: 17-Apr-2006

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 350492
Product name pcDNA3.1/CAT

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.

Form
Solid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available

Water solubility	No data available

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

invitrogen™

Material Safety Data Sheet

Revision Date: 27-Mar-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code A10562
Product name pENTR™ 11 Dual Selection Vector

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes No information available
Skin No information available

3. HAZARDS IDENTIFICATION

Inhalation No information available
Ingestion No information available

Specific effects

Carcinogenic effects No information available
Mutagenic effects No information available
Reproductive toxicity No information available
Sensitization No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact Wash off immediately with plenty of water
Eye contact Rinse thoroughly with plenty of water, also under the eyelids.
Ingestion Never give anything by mouth to an unconscious person
Inhalation Move to fresh air
Notes to physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media Dry chemical
Special protective equipment for firefighters Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions Use personal protective equipment
Methods for cleaning up Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling No special handling advice required
Storage Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits
Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.

Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

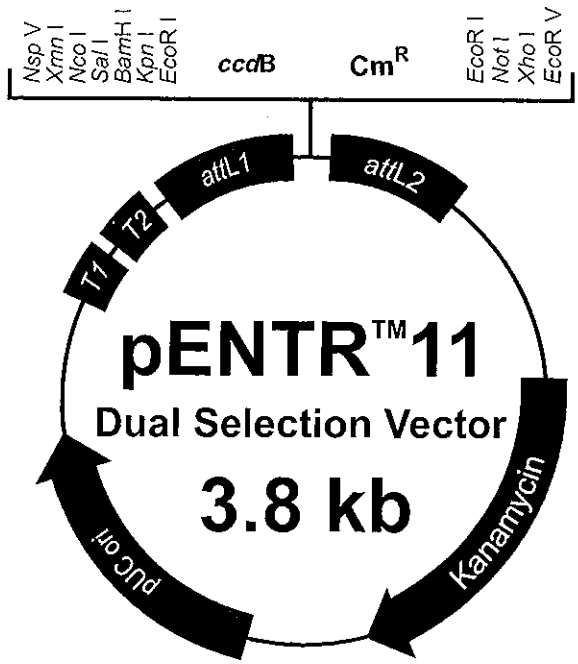
This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet



Comments for pENTR™11
3781 nucleotides

*rrn*B T1 transcription termination sequence: bases 106-149
*rrn*B T2 transcription termination sequence: bases 281-308
*att*L1: bases 358-457 (complementary strand)
*ccd*B gene: bases 570-872 (complementary strand)
Chloramphenicol resistance gene (Cm^R): bases 1217-1872 (complementary strand)
*att*L2: bases 2010-2109
Kanamycin resistance gene: bases 2232-3041
pUC origin: bases 3105-3778

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Material Safety Data Sheet

Revision Date: 09-Nov-2006

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Contact manufacturer
INVITROGEN CORPORATON
1600 FARADAY AVENUE
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	5-10

The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health.

Form
Liquid

Personal protective equipment

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability	Stable.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

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Material Safety Data Sheet

Revision Date: 11-Feb-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18290015
Product name ELECTROMAX DH10B COMP CELLS

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
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++1 716 774 6700

INVITROGEN CORPORATION NEW ZEALAND LIMITED
18 - 24 BOTHA ROAD
PENROSE
AUCKLAND 1006
NEW ZEALAND
0800-600-200

INVITROGEN AUSTRALIA PTY LIMITED
2A/14 LIONEL ROAD
MOUNT WAVERLY VIC 3149
AUSTRALIA
1-800-331-627

Emergency Number (Spills, Exposures): 301/431-8585 (24-hour)

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
Caution - substance not yet fully tested. To our knowledge, the hazards of this material have not been thoroughly investigated. We recommend handling all chemicals with caution

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Suspension

Respiratory protection	In case of insufficient ventilation wear suitable respiratory equipment
Hand protection	Protective gloves
Eye protection	Safety glasses with side-shields
Skin and body protection	Lightweight protective clothing.
Hygiene measures	Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls	Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form	Suspension

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	soluble	

10. STABILITY AND REACTIVITY

Stability	Stable under normal conditions.
Materials to avoid	No information available
Hazardous decomposition products	No information available
Polymerization	Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes	May cause eye irritation with susceptible persons.
Skin	May cause skin irritation in susceptible persons.
Inhalation	May cause irritation of respiratory tract.
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects — No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

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MAX Efficiency® DH10B™ Competent Cells

Cat. No. 18297-010

Size: 1 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:

MAX Efficiency® DH10B™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan(1). The presence of the *mcr*A genotypic marker and the deletion of *mcr*BC and *mrr* makes this strain suitable for cloning DNA that contains methylcytosine and methyladenine (2,3,4). Therefore, genomic DNA, both prokaryotic and eukaryotic, can be cloned efficiently in DH10B™ (2,3,4,5). Plasmid rescue is also more efficiently performed with DH10B™ (6). MAX Efficiency® DH10B™ is resistant to the effects of ligase and ligase buffer and thus can tolerate the addition of small amounts of undiluted ligation reactions (see Note 4).

These cells are suitable for the construction of gene banks or for the generation of cDNA libraries using plasmid- derived vectors. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the ß-galactosidase gene from pUC or similar vectors and therefore can be used for blue/white screening of colonies on bacterial plates containing X-gal or Bluo-gal.

Genotype

F⁻ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lacZ*ΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ(*ara, leu*)7697 *gal*U *gal*K λ⁻ *rps*L *nup*G /pMON14272 / pMON7124

Component	Amount per Vial
DH10B™ Competent Cells	200 µl
pUC19 DNA (0.01 µg/ml)	100 µl

Growth of Transformants for Plasmid Preparations:

MAX Efficiency® DH10B™ Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB (9). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes:

1. Falcon® 2059 tubes or other similarly shaped 17 × 100 mm polypropylene tubes are required for optimal transformation efficiency. Microcentrifuge tubes (1.5 ml) can be used but the transformation efficiency will be reduced 3- to 10-fold.
2. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately 2-fold.
3. Media other than S.O.C. Medium can be used but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of 2- to 3-fold (8).
4. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid such as pUC19. Our data indicates that DH10B™ cells can tolerate the addition of up to 1 µl (5-50 ng) of an undiluted ligation reaction (standard 20 µl ligation reaction using 1 unit of T4 DNA ligase and 1X ligation buffer) without a significant loss in transformation efficiency (approximately 2-fold). We have observed that the cells begin to saturate with 10-50 ng of DNA (7).
5. Generally, transformation efficiencies will be 10- to 100-fold lower for cDNA than for an intact control plasmid such as pUC19. The amount of cDNA used in a 100 µl transformation should be 1-5 ng in 5 µl or less.

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MAX Efficiency® DH5α™ Competent Cells

Cat. No. 18258-012

Size: 1 ml
Store at -70°C.
Do not store in liquid nitrogen.

Description:

MAX Efficiency® DH5α™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). These cells are suitable for the construction of gene banks or for the generation of cDNA libraries using plasmid-derived vectors. The ϕ80d*lac*ZΔM15 marker provides α-complementation of the β-galactosidase gene from pUC or similar vectors and, therefore, can be used for blue/white screening of colonies on bacterial plates containing Bluo-gal or X-gal. DH5α™ is capable of being transformed efficiently with large plasmids, and can also serve as a host for the M13mp cloning vectors if a lawn of DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101 or JM107 is provided to allow plaque formation.

Genotype

F- ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (r_K-, m_K+) *pho*A *sup*E44 λ- *thi*-1 *gyr*A96 *rel*A1

Component	Amount per Vial
DH5α™ Competent Cells	200 µl
pUC19 DNA (0.01 µg/ml)	100 µl

Quality Control: MAX Efficiency® DH5α™ Competent Cells consistently yield > 1.0×10^9 transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate > 1×10^6 ampicillin-resistant colonies in a 100-µl reaction.

For technical support, email tech_support@invitrogen.com
For country-specific contact information, visit *www.invitrogen.com*

Growth of Transformants for Plasmid Preparations:

DH5α™ Competent Cells which have been transformed with pUC-based plasmids should be grown at 37°C overnight in TB(2). A 100-ml growth in a 500-ml baffled shake flask will yield approximately 1 mg of pUC19 DNA.

Notes:

1. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately two-fold.
2. Media other than S.O.C. Medium can be used, but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of two- to three-fold (4).
3. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. Ligation reactions should be diluted 5-fold prior to using the DNA in a transformation. Only 1 µl of this dilution should be used. A standard ligation reaction (20 µl) normally contains 100-1000 ng of DNA. Therefore, the addition of 1 µl of diluted DNA will result in adding 1 to 10 ng of ligated DNA to the cells. We have observed that the cells begin to saturate with 10-50 ng of DNA (3). Also our data show that the 5-fold dilution of ligation mixtures results in more efficient transformation (3,4).
4. MAX Efficiency® DH5α™ can support the replication of M13mp vectors. However, DH5α™ is F⁻ and cannot support plaque formation. Therefore, log phase DH5α-FT™, DH5αF'™, DH5αF'IQ™, JM101 or JM107 cells must be added to the top agar which should contain X-gal (Cat. No. 15520-034) or Bluo-gal, final concentration 50 µg/ml, and IPTG (Cat. No. 15529-019), final concentration 1 mM. The competent cells should be added to top agar after lawn cells, IPTG and Bluo-gal or X-gal have been added. Incubation at 37°C for 1 hour is not required after addition of S.O.C. Medium.

MATERIAL SAFETY DATA SHEET

Revised 10/30/03
Replaces 9/05/03
Printed 10/30/03

ME DH5A T1 PHAGE RESISTANT COMPETENT CELLS
INVITROGEN CORPORATION
MSDS ID: 12034

1. PRODUCT AND COMPANY INFORMATION

INVITROGEN CORPORATION
1600 FARADAY AVE.
CARLSBAD, CA 92008
760/603-7200

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716/774-6700

INVITROGEN CORPORATION
3 FOUNTAIN DR.
INCHINNAN BUSINESS PARK
PAISLEY, PA4 9RF
SCOTLAND
44-141 814-6100

INVITROGEN CORPORATION
P.O. BOX 12-502
PENROSE
AUCKLAND 1135
NEW ZEALAND
64-9-579-3024

INVITROGEN CORPORATION
2270 INDUSTRIAL ST.
BURLINGTON, ONT
CANADA L7P 1A1
905/335-2255

EMERGENCY NUMBER (SPILLS, EXPOSURES): 301/431-8585 (24 HOUR)
800/451-8346 (24 HOUR)
NON-EMERGENCY INFORMATION: 800/955-6288

Product Name: ME DH5A T1 PHAGE RESISTANT COMPETENT CELLS
Stock Number: 12034

NOTE: If this product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

Product Use:
These products are for laboratory research use only and are not intended for human or animal diagnostics, therpeutic, or other clinical uses.

Synonyms:
Not available.

2. COMPOSITION, INFORMATION ON INGREDIENTS

The following list shows components of this product classified as hazardous based on physical properties and health effects:

Component	CAS No.	Percent
DIMETHYL SULFOXIDE	67-68-5	1 - 5

3. HAZARDS IDENTIFICATION

********** EMERGENCY OVERVIEW **********
Warning!
Irritant.
Harmful if absorbed.
****

Potential Health Effects:
Eye:
Can cause moderate irritation, tearing and reddening, but not likely to permanently injure eye tissue.

Skin:
Can cause moderate skin irritation, defatting, and dermatitis. Not likely to cause permanent damage.
Upon prolonged or repeated exposure, harmful if absorbed through the skin. May cause minor systemic damage.

Inhalation:
Can cause moderate respiratory irritation, dizziness, weakness, fatigue, nausea and headache.
No toxicity expected from inhalation.

Ingestion:
Irritating to mouth, throat, and stomach. Can cause abdominal discomfort, nausea, vomiting and diarrhea.

Chronic:
No data on cancer.

4. FIRST AID MEASURES

Eye:
Flush eyes with plenty of water for at least 20 minutes retracting eyelids often. Tilt the head to prevent chemical from transferring to the uncontaminated eye. Get immediate medical attention.

Skin:
Wash with soap and water. Get medical attention if irritation develops or persists.

Inhalation:
Remove to fresh air. If breathing is difficult, have a trained individual administer oxygen. If not breathing, give artificial respiration and have a trained individual administer oxygen. Get medical attention immediately.

Ingestion:
Do not induce vomiting and seek medical attention immediately. Drink two

4. FIRST AID MEASURES (CONT.)

glasses of water or milk to dilute. Provide medical care provider with this MSDS.

Note To Physician:
Treat symptomatically.

5. FIRE FIGHTING MEASURES

Flashpoint Deg C:
Not available.

Upper Flammable Limit %:
Not available.

Lower Flammable Limit %:
Not available.

Autoignition Temperature Deg C:
Not available.

Extinguishing Media:
Use alcohol resistant foam, carbon dioxide, dry chemical, or water spray when fighting fires. Water or foam may cause frothing if liquid is burning but it still may be a useful extinguishing agent if carefully applied to the fire. Do not direct a water stream directly into the hot burning liquid. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Firefighting Techniques/Equipment:
Do not enter fire area without proper protection including self-contained breathing apparatus and full protective equipment. Fight fire from a safe distance and a protected location due to the potential of hazardous vapors and decomposition products.

Hazardous Combustion Products:
Carbon dioxide Carbon monoxide Sulfur containing gases

6. ACCIDENTAL RELEASE MEASURES

Accidental releases may be subject to special reporting requirements and other regulatory mandates. Refer to Section 8 for personal protection equipment recommendations.

6. ACCIDENTAL RELEASE MEASURES (CONT.)

Spill Cleanup:
Exposure to the spilled material may be irritating or harmful. Follow personal protective equipment recommendations found in Section VIII of this MSDS. Additional precautions may be necessary based on special circumstances created by the spill including; the material spilled, the quantity of the spill, the area in which the spill occurred. Also consider the expertise of employees in the area responding to the spill.
Ventilate the contaminated area.
Absorb spill. Common absorbent materials should be effective. Deposit in appropriate containers for removal and disposal.

7. HANDLING AND STORAGE

Storage of some materials is regulated by federal, state, and/or local laws.

Storage Pressure:
Ambient

Handling Procedures:
Harmful or irritating material. Avoid contacting and avoid breathing the material. Use only in a well ventilated area.
Keep closed or covered when not in use.

Storage Procedures:
Store in a cool dry ventilated location. Isolate from incompatible materials and conditions. Keep container(s) closed.
Suitable for most general chemical storage areas.

8. EXPOSURE CONTROLS, PERSONAL PROTECTION

Exposure Limits:

Component	OSHA PEL (ppm)	AGCIH TWA (ppm)
DIMETHYL SULFOXIDE	Not established.	Not established.

Engineering Controls:
Local exhaust ventilation or other engineering controls are normally required when handling or using this product to avoid overexposure.

Personal Protective Equipment:

Eye:
Safety glasses should be the minimum eye protection.
Wear chemically resistant safety glasses with side shields when handling this product. Wear additional eye protection such as chemical splash

8. EXPOSURE CONTROLS, PERSONAL PROTECTION (CONT.)

goggles and/or face shield when the possibility exists for eye contact with splashing or spraying liquid, or airborne material. Do not wear contact lenses. Have an eye wash station available.

Skin:
Avoid skin contact by wearing chemically resistant gloves, an apron and other protective equipment depending upon conditions of use. Inspect gloves for chemical break-through and replace at regular intervals. Clean protective equipment regularly. Wash hands and other exposed areas with mild soap and water before eating, drinking, and when leaving work. Gloves should be used as minimum hand protection.

Respiratory:
Use supplied-air respiratory equipment as required.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance/physical state:	Liquid solution / suspension
Odor:	No odor.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
	Not established.
Specific Gravity/Density:	Not established.
Octanol/water Partition Coeff:	Not established.
Volatiles:	Not established.
Evaporation Rate:	Not established.
Viscosity:	Not established.

10. STABILITY AND REACTIVITY

Stability:
Stable under normal conditions.

Conditions to Avoid:
Strong oxidizing agents. Temperatures above the high flash point of this combustible material in combination with sparks, open flames, or other sources of ignition. Strong alkalies. DMSO undergoes a violent exothermic reaction on mixing with copper wool and trichloroacetic acid. On mixing with potassium permanganate it will flash instantaneously. It reacts violently with: acid halides, cyanuric chloride, silicon tetrachloride, phosphorus trichloride and trioxide, thionyl chloride, magnesium perchlorate, silver fluoride, methyl bromide, iodine pentafluoride, nitrogen periodate, diborane, sodium hydride, perchloric and periodic acids. When heated above its boiling point, DMSO

10. STABILITY AND REACTIVITY (CONT.)

degrades giving off formaldehyde, methyl mercaptan, and sulfur dioxide.

Hazardous Decomposition Products:
Carbon monoxide. Carbon dioxide. Sulfur containing gases.

Hazardous Polymerization:
Hazardous polymerization will not occur.

11. TOXICOLOGICAL INFORMATION

Acute Toxicity:

Dermal/Skin:
DIMETHYL SULFOXIDE: 40 GM/KG

Inhalation/Respiratory:
Not determined.

Oral/Ingestion:
DIMETHYL SULFOXIDE: 14,500 MG/KG

Target Organs: Blood. Eyes. Skin.

Carcinogenicity:

NTP:
Not tested.

IARC:
Not listed.

OSHA:
Not regulated.

Other Toxicological Information

12. Ecological Information

Ecotoxicological Information: No ecological information available.

Environmental Fate (Degradation, Transformation, and Persistence):
Bioconcentration is not expected to occur.
Biodegrades slowly.

13. DISPOSAL CONSIDERATIONS

Regulatory Information:
Not applicable.

Disposal Method:
Clean up and dispose of waste in accordance with all federal, state, and local environmental regulations.
Dispose of by incineration following Federal, State, Local, or Provincial regulations.

14. TRANSPORT INFORMATION

Proper Shipping Name: Not Determined.
Subsidiary Hazards:

15. REGULATORY INFORMATION

UNITED STATES:

TSCA:
This product is solely for research and development purposes only and may not be used, processed or distributed for a commercial purpose. It may only be handled by technically qualified individuals.

Prop 65 Listed Chemicals: PROP 65 PERCENT
No Prop 65 Chemicals.

No 313 Chemicals

CANADA:

DSL/NDSL:
Not determined.

COMPONENT	WHMIS Classification
DIMETHYL SULFOXIDE	D2B

EUROPEAN UNION:

PRODUCT RISK PHRASES:
None assigned.

PRODUCT SAFETY PHRASES:
Not applicable.

PRODUCT CLASSIFICATION: