The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents 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).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards 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.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Paula Foster**
DEPARTMENT:	**Imaging/Medical Physics**
ADDRESS:	**Robarts Research Institute 100 perth dr Rom2276**
PHONE NUMBER:	**24040**
EMERGENCY PHONE NUMBER(S):	**24040**
EMAIL:	**pfoster@imaging.robarts.ca**

Location of experimental work to be carried out :

Building :	**RRI**	Room(s):	**2267,2222, 3T MRI,**
Building :	**UH**	Room(s):	**L1H1.5**
Building :		Room(s):	

***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 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **CIHR, NCIC, OCIR, MSSOC, ORF**
GRANT TITLE(S): **See attached documents**
UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Yuhua Chen	**ychen@robarts.ca**	**attached**
Catherin McFadden	**mcfadden@imaging.robarts.ca**	**attached**
Amanda Hemilton	**hamilton@imaging.robarts.ca**	**attached**
Emeline Ribot	**eribot@imaging.robarts.ca**	**attached**
Gabrielle Siegers	**gsiegers@imaging.robarts.ca**	**attached**
Laura Gonzalez Lara	**gonzalez@imaging.robarts.ca**	**attached**

Christiane Mallett	cmall@imaging.robarts.ca	attached
Vasiliki Economopoulos	vecon@imaging.robarts.ca	attached
Mariama Henry	mhenry@imaging.robarts.ca	attached
Matthew Lowerison	mloweri@imaging.robarts.ca	attached
Grag Dekaban	dekaban@robarts.ca	attached
Yonathan Araya	yaraya@uwo.ca	attached

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

All cell lines will be treated in the same fashion.
Cells will be subcultured aseptically in a biological cabinet. They will be labeled with super paramagnetic iron oxide particles , if labeling is successful, then the cells will be injected into the animals. The remaining cells will be disposed of bleach. The methods of injections include subcutneous, intranodal (lymph node), intracardiac, and intravenous. The injection will be performed in the room 2276 at RRI. The mice will then be housed at ACVS and periodically scanned with MRI at RRI 3T room or University Hospital's 1.5T MRI facility. After reaching the end points, animals will be sacrificed, the tissues of interest removed, and placed into formalin for futher ex vivo scaning or tissue processing. Remaining tissues will be disposed of at the ACVS facility for incineration.

Pertussis Toxin:
The pertussis toxin will be administered to mice with experimental autoimmune encephalomyelitis(EAE). After an intraperitoneal injection, pertussis toxin will cause proliferation of Tcells , which is imprortant in the induction process of EAE in mice. The toxin is missing a subunit resulting in extremely low toxicity in humans, is injectected by way of intraperitoneal injection. The injection will be done by an experienced member of the lab. An SOP has been created. Animals will be housed and imaged as described above .

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

The research performed in the Foster lab is focused on the use of MRI to detect and track cells in preclincial models of disease. Various strategies for labeling cells with iron oxide nanoparticles are developed in the lab. A variety of cell types have been labeled (cancer cells, immune cells, stem cells) for use in these projects. Iron labeled cells are injected or transplanted into mouse models of disease and MRI is used to monitor the cell survival, location and persistence over time (typcially weeks). One focus in the lab is tracking cancer cell metastasis. Another is tracking the fate of stem cells tranplanted in a model of multiple sclerosis. At the end of the imaging experiments the mice are euthanized and the tissues of interest are fixed and prepared for histological analysis to confirm the MRI findings.

1.0 Microorganisms

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

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☐ NO

If YES, please give the name of the species ______________________________

What is the origin of the microorganism(s)? ______________________________

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

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	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
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No		

****Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: ______________________________

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☒ YES ☐ NO
(If NO, please proceed to Section 3.0)

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

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☒ Yes ☐ No	**human blood**	Not applicable
Rodent	☐ Yes ☒ No		
Non-human primate	☐ Yes ☒ No		
Other (specify)	☐ Yes ☒ No		

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☒ Yes ☐ No	**attached**	**attached**	**attached**
Rodent	☒ Yes ☐ No	**attached**	**attached**	**attached**
Non-human primate	☐ Yes ☒ No			
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 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

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

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

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid	**Various health donors**	☐ Yes ☒ Unknown	**n/a**	☐1 ☒2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid	**n/a**	☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)	**n/a**	☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)	**n/a**	Not Applicable		Not Applicable

Additional Comments: _____

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 Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?
	see attached sheet					

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☒ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☒ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☒ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☒ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

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

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

6.4 How will the biological agent be administered?

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

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☒ YES ☐ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used **Nu/Nu(nude) , SCID and C57B1/6 mice**

7.3 AUS protocol # **2900-042, 2010-210**

7.4 List the location(s) for the animal experimentation and housing. **RRI 2276 and ACVS**

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☒ YES, specify: **All**

7.6 Will the agent(s) be shed by the animal:
☐ YES ☒ NO, please justify: **The bio-agents are injected into the animal. That will inducte the animal cancer model and they won't be shed by the animal.**

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☒ NO

8.3 If **YES**, please specify the animal(s) used:

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

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☒ YES ☐ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s) **Pertussis toxin**
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone **18ug/kg. no information for human**

9.4 How much of the toxin or hormone is handled at one time*? **200ng /each mouse**

9.5 How much of the toxin or hormone is stored*? **50-70ug**

9.6 Will any biological toxins or hormones be used in live animals? ☒ YES ☐ NO
If **YES**, Please provide details: **Pertussis toxin**

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

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

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

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

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

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

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

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☒ YES, country of origin **Germany** ☐ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☒ YES ☐ NO

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

12.4 Has the import permit been sent to OHS? ☒ YES, please provide permit # **P-11-0060** ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Paula Foster** ***Date:*** **Nov 14.2011**

14.0 Containment Levels

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

14.2 Has the facility been certified by OHS for this level of containment?
☒ YES, location and date of most recent biosafety inspection: **Rm#2222, Rm#2276**
☐ NO, please certify
☐ NOT REQUIRED for Level 1 containment

Lab has moved needs to be recertified.

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☐ YES ☒ NO
If **YES** please describe:

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
The injection will be done by a experienced member of Lab. An SOP has been created.

15.3 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.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name **Paula Foster** ***Date:*** **Nov 14 2011**

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

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

Special Conditions of Approval:

Foster Lab Training Cetrificate Inventory

	BioSafety Training	WHMIS Training	Waste Mamagement Safety Training	Radiation Safety Training
Amanda Hamilton	10-Feb-11	19-Jan-11	26-Oct-11	
Catherine Ramsay	20-May-11	19-Mar-09	20-May-09	
Christiane Mallett	13-Jun-06	01-Apr-11		
Emeline Ribot	07-May-09	03/04/09	05-Jul-09	
Gabrielle Siegers	24-Sep-10	04-Oct-11	27-Sep-10	16-Sep-10
Jonatan Snir		05-May-11	04-May-11	
Laura Gonzalez Lara	07-Jan-11	07-Jan-10		
Mariama Henry	26-Oct-11	26-Sep-11		
Vasiliki Economopoulos	04-Oct-08	22-Sep-11	26-Sep-11	
Yuhua Chen	21-Nov-07	03-Apr-11	08-Mar-11	
Grag Dekaban	02/06/05	~~16/10/2007~~ 29/03/2011		
Metthew Lowerison	26-Aug-11	03-Aug-11	22-Sep-11	
Yonathan Araya	24-Nov-11	27-Jan-11	16-Nov-11	

18/01/2012

Section (2.3) cell Lines

Cell line will be numbered. These numbers will be indicated on the form due to limited space on the form.

Name Type	Supplier
(1) THP-1 (human)	ATCC(TIB-202)
(2) B16F10 (Rodent)	Anne Chambers LRCC (collaboration)
(3) Glioma-261 (Rodent)	National Cancer institute (NCI)
(4) Glioma-261RFP (Rodent)	NCI
(5) FaDu Luc2.11 (Human)	Becton Dickinson Technologies (Collaboration)(Purchase from Attc(HTB-43) before modification done by BD)
(6) NDA-MB-231-231PA(human)	Anne Chambers LRCC(collaboration) (HTB-26)
(7) MDA-MB-231BR(human)	Anne Chambers LRCC (HTB-26)
(8) MDA-MB-231-luc-D3H2LN)	Anne Chambers LRCC(HTB-26)
(9) MDA-MB-435	Anne Chambers LRCC NCIDTP60
(10) MDA-MB-231BR eGFP	Dr. Brian Rutt's lab (collaboration) (HTB-26)
(11) MDA-MB-231 with mms6 pcDNA	Dr. Brian Rutt's lab (HTB-26)
(12) MDA-MB-231 with magA pcDNA	Dr. Brian Rutt's lab (HTB-26)
(13) MDA-MB-231 with pcDNA	Dr. Brian Rutt's lab (HTB-26)
(14) MDA-MB-231BR eGFP with mms6 pDsRed	Dr. Brian Rutt's lab (HTB-26)
(15) MDA-MB-231BR eGFP with magA pDsRed	Dr. Brian Rutt's lab(HTB-26)
(16) MDA-MB-231BR eGFP with mms6-DsRed pDsRed	Dr. Brian Rutt's lab(HTB-26)
(17) MDA-MB-231BR eGFP with magA-mms6 pDsRed	Dr. Brian Rutt's lab(HTB-26)
(18) MDA-MB-231BR eGFP with magA-DsRed pDsRed	Dr. Brian Rutt's lab(HTB-26)
(19) MDA-MB-231BR eGFP with pDsRed	Dr. Brian Rutt's lab(HTB-26)
(20)KHYG-1(human)	U of T Dr. Keating lab (collaboration (JCRB0156)
(21) C4-2B (human)	ViroMed Laboratories
(22) PC-3 (human)	NCI
(23) Melanoma A2058(human)	Cedarlane (Attc: CRL-11147)
(24) EM-2 Ph(+) leukemia (human)	Dr. Keating lab (Attc:135)
(25) EM-2eGFPluc Ph(+) leukemia (huma)	T.Felizardo (Attc:135)
(26) MEC-1 B-CLL (human)	J.Schueler, Germany (Acc 479)
(27) TMD2 B-CLL S. (human)	Tohda, Japan information is attached
(28) RAJI Burkitt's lymphoma (human)	UHN collaborator (Attc CCL-86)
(29) RAJIeGFPluc Burkitt's lymphoma (human)	Dr. T.Felizardo (Attc CCL-86)
(30) K562 Ph(+) leukemia (human)	Dr. T.Felizardo (Attc CCL-86)
(31) K562eGFPluc Ph(+) leukemia (human)	Dr. T. Felizardo (Attc CCL-86)

Primary Cell

(1) GDTs (human)	Various healthy donors
(2) Human mesenchymal stem cells	U of T Dr. Keating lab (information is attached)
(3)Horse mesenchymal stem cells	University of Guelph (information is attached)

Section (4.2) Genetic modification(s) involving plasmids

Plasmid Name	Gene Transfected	Results
(1) PCMV-DSRED Express	RFP	RFP expressed
(2) PGL4.1 Luc Luciferase expressed	Luc	Luciferase expressed
(3) PCC-EGFP-minCMV-hPGK-Luc lentivra vector	Luc lentiviral vector	Luciferase expressed
(4) PCDNA3.1(+) 231PA	mms6 or magA	Gene expression
(5) PEYFP-C1	magA, YFP	Gene expression
(6) pDsRed monomer-Hyg-N1 (231Br)	mms6 or magA	Gene expression

All the modifications were done by our collaborators in their lab.

Grant project titles

Early and Targeted Detection of Metastatic Cancer in the Lymphatic System Using Cellular Magnetic Resonance Imaging and Nanotechnology(NCIC)

One Millimetre Cancer Challenge (1mmCC), "Tracking Cancer(OICR) Vaccines"

The Use of Cellular MRI to Evaluate Stem Cell Transplantation in a Model of Multiple Sclerosis(MSSOC)

Ontario pre-clinical imaging consortium ----Foster/Rutt/McKenzie (ORF)

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PERTUSSIS TOXIN
(ISLET-ACTIVATING PROTEIN)

Pertussis toxin is the major protein toxin produced by virulent strains of *Bordetella pertussis*, the organism that causes whooping cough.[1] As revealed by poly-acrylamide gel electrophoresis, the purified protein consists of five dissimilar subunits: S-1 (molecular weight 28,000 daltons), S-2 (23,000), S-3 (22,000), S-4 (11,700) and S-5 (9,300), in a molar ratio of 1:1:1:2:1. S-1 (A protomer) is responsible for the enzymic activity of the toxin. Together, S-2, S-3, S-4 and S-5 comprise the B oligomer, responsible for binding the toxin to the cell surface.[2]

Pertussis toxin elicits a variety of physiological responses, including induction of lymphocytosis, stimulation of insulin secretion, and sensitization to histamine. It has thus been variously referred to in the literature as lymphocytosis-promotingfactor,[3,4] islet-activating protein,[5] histamine-sensitizing factor [6] and pertussigen.[7] The toxin also acts as a hemagglutinin,[8] and serves as a protective antigen in mice against challenge with *B. pertussis*.[3,9,10,11] Further, treatment of Chinese hamster ovary cells with purified toxin results in a unique clustered growth pattern which provides a useful *in vitro* assay for the toxin.[12]

Pertussis toxin has been found to catalyze the ADP-ribosylation of the G_i regulatory component of adenylate cyclase,[13,14] and it is this action that is believed to be responsible for the various physiological and cellular effects of the toxin. Studies with rat heart cells,[15] pancreatic islets[16,17] and G6 glioma cells[18] have shown that treatment with pertussis toxin potentiates cyclic AMP accumulation in response to normal stimuli. Further, cells treated with the toxin fail to respond to agents that normally block cyclic AMP accumulation.[19] Thus, pertussis toxin has become a valuable tool in the study of the regulation of adenylate cyclase. The action of pertussis toxin on the G_i component of adenylate cyclase has also been found to inhibit various metabolic responses of neutrophils to chemotactic factors, implying a role for G_i in these functions as well.[20,21,22] Recently, it has been found that pertussis toxin catalyzes the ADP-ribosylation of transducin, a guanine nucleotide-binding regulatory protein that mediates activation of a retinal cyclic GMP-selective phosphodiesterase.[23,24]

Pertussis toxin from List Biological Laboratories is isolated from *Bordetella pertussis* by a modification of the method of Cowell *et al.*[25] This preparation is highly purified, migrating as five distinct bands as described by Tamura *et al.* [2] when run on 15% polyacrylamide SDS-urea gels according to the method of Laemmli.[26] It contains no detectable fimbrial hemagglutinin on gels, and no measurable *B. pertussis* adenylate cyclase activity when assayed by the method of Wolff *et al.*[27] in the absence or presence of 1 µmolar calmodulin. Each lot is tested for its activity in the CHO cell assay as described by Hewlett *et al.*[12]

Pertussis toxin is supplied in three formulations. The first is frozen in 50% glycerol, 0.05 M Tris, pH 7.5, 0.01 M glycine, 0.5 M NaCl. The second is lyophilized and when reconstituted with 0.5 ml of distilled water contains 50.0 µg of protein in 0.01 M sodium phosphate buffer, pH 7.0, with 0.05 M sodium chloride. Finally, pertussis toxin is also available lyophilized in a salt-free form. A detailed lot analysis documenting purity and biological activity plus complete instructions on reconstitution and storage accompany each shipment.

This product is intended for research purposes only and is not for use in humans. For further information, please contact List Biological Laboratories, Inc.

Ordering Information

Product No.	Description	Size
179A	Pertussis Toxin (frozen)	50.0 µg
179B	Persussis Toxin (frozen)	200.0 µg
180	Pertussis Toxin (lyophilized)	50.0 µg
181	Pertussis Toxin (lyophilized, salt-free)	50.0 µg

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Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number:	**HTB-43™** Order this Item	**Price:**	**$294.00**
Designations:	FaDu	**Depositors:**	SR Rangan
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
Source:	**Organ:** pharynx **Disease:** squamous cell 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.		

Related Cell Culture Products

Applications:	transfection host(Roche FuGENE® Transfection Reagents)
Tumorigenic:	Yes
Cytogenetic Analysis:	(P16) hypodiploid to hypertriploid with modal number = 64
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 2 PGM1, 2 PGM3, 1
Age:	56 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The FaDu line was established in 1968 from a punch biopsy of an hypopharyngeal tumor removed from a Hindu patient. The established line was found to contain bundles of tonofilaments in the cell cytoplasm and desmosomal regions were prominent at cell boundaries.
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%. **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 2 to 3 times per week Remove medium, and rinse with 0.25% trypsin, 0.03% EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.
Preservation:	Culture medium, 95%; DMSO, 5%

Product Description

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number:	**TIB-202™** Order this Item	**Price:**	**$244.00**
Designations:	THP-1	**Depositors:**	S Tsuchiya
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	suspension
Organism:	*Homo sapiens* (human)	**Morphology:**	monocyte
Source:	**Organ:** peripheral blood **Disease:** acute monocytic leukemia **Cell Type:** monocyte;		
Cellular Products:	lysozyme [58053]		
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)		
Receptors:	complement (C3), expressed [58053] Fc, expressed		
Antigen Expression:	HLA A2, A9, B5, DRw1, DRw2 [58053]		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,13 D13S317: 13 D16S539: 11,12 D5S818: 11,12 D7S820: 10 THO1: 8,9.3 TPOX: 8,11 vWA: 16		
Age:	1 year infant		
Gender:	male		
Comments:	The cells are phagocytic (for both latex beads and sensitized erythrocytes) and lack surface and cytoplasmic immunoglobulin. [58053] Monocytic differentiation can be induced with the phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA). [2193]		
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: 2-mercaptoethanol to a final concentration of 0.05 mM; fetal bovine serum to a final concentration of 10%. **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C		

Product Description

Before submitting an order you will be asked to read and accept the terms and conditions of ATCC's Material Transfer Agreement or, in certain cases, an MTA specified by the depositing institution.
Customers in Europe, Australia, Canada, China, Hong Kong, India, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number:	**HTB-26™** Order this Item	**Price:**	**$244.00**
Designations:	MDA-MB-231	**Depositors:**	R Cailleau
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial		
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)		
Receptors:	epidermal growth factor (EGF), expressed transforming growth factor alpha (TGF alpha), expressed		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X CSF1PO: 12,13 D13S317: 13 D16S539: 12 D5S818: 12 D7S820: 8,9 THO1: 7,9.3 TPOX: 8,9 vWA: 15,18		
Cytogenetic Analysis:	The cell line is aneuploid female (modal number = 64, range = 52 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Eleven stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.		
Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 1-2 PGM1, 1-2 PGM3, 1		
Age:	51 years adult		

BD	FaDu*Luc*2.11	CONFIDENTIAL
Date: October 30, 2007	Created by: Scott Kaestner	Page 1 of 1

Cell Line	FaDuLuc2.11
Description	The FaDu (human, pharynx squamous cell carcinoma) cell line was established from a punch biopsy of a hypo pharyngeal tumor removed from a Caucasian male. This cell line is adherent and tumorigenic in nude mice. The cell line was transfected with a plasmid containing the Luc2 and Hygromycin resistance genes making the cells bioluminescent in the presence of Luciferin and resistant to Hygromycin. A FaDuLuc2.11 cell contains about 0.3 pg of Luciferase. FaDu cells stain positive with Pancytokeratin immuno histochemical staining. FaDu cells over express Epithelial Growth Factor Receptor.
Media and sub cultivation	RPMI-1640 with L-glutamine, 10% Fetal Bovine Serum, 10,000 IU/ml penicillin, 10,000µg/ml streptomycin, and 150 µgs/ml Hygromycin. Cryoprotectant media is the same as above supplemented with 5% DMSO. A sub cultivation ratio of 1:3 to 1:6 is best for this cell line and is compatible with a bi-weekly media replacement and passaging regiment.
Tumorigenicity	Primary subcutaneous tumor: 5/5 (100%), $1.0x10^6$ cells inoculated, Bioluminescent detection at day 7, palpable at day 14. Intranodal tumor: 11/19 (58%), $5.0x10^4$ cells inoculated, Bioluminescent detection at day 1 and 5.
MTA and other IP	The original FaDu cell line was purchased from ATCC and may be subject to terms from the Material transfer agreement at time of purchase. The Promega pGL4.14 vector is the primary backbone of the plasmid used for transfection of the FaDu cell line and some genes may be patent protected.

NCI-FREDERICK CANCER
DCT TUMOR REPOSITORY

AVAILABLE INFORMATION ON TRANSPLANTABLE TUMORS OR CELL LINES

DESIGNATION:	**PC-3/M**
VIAL DESIGNATION:	0502392
SPECIES:	HUMAN
TUMOR ORIGIN:	PROSTATE
HISTOLOGIC TYPE:	CARCINOMA

CELL CULTURE INFORMATION:

GROWTH MEDIUM:	RPMI 1640 + 10%FBS + 2 mM L-Glutamine
MODE OF GROWTH:	Adherent
METHOD OF HARVEST:	Trypsin/EDTA
SPLIT RATIO:	1:20 Weekly
DOUBLING TIME:	60 Hours

TUMOR PROFILE INFORMATION:

HOST STRAIN:	ATHYMIC NUDE
TISSUE IMPLANTED:	N/A
ROUTE:	N/A
SUSPENDING MEDIUM:	N/A
OPTIMAL DAY:	N/A
HOST MEDIAN SURVIVAL TIME:	N/A
METASTATIC CHARACTERISTICS:	N/A
HISTOPATHOLOGY:	N/A

SOURCE:	Dr. Edward Kaighn
REFERENCE:	Ohnuki, Y., Marnell, M., Babcock, M., Lechner, J., Kaighn, E.; Chromosomal Analysis of Human Prostatic Adenocarcinoma Cell Lines; Cancer Research 40: 524-534; March 1980.
CRYOPRESERVED:	10/27/87
VIRAL PROFILE:	NEGATIVE
OTHER INFORMATION:	The subline, PC-3/M, had a similar karyotype and retained the parental PC-3 markers. PC-3/M had a more restricted chromosomal frequency distribution range. Nearly 73% of the PC-3/M cells examined had 60 or 61 chromosomes in contrast to the wide distribution seen in PC-3. Silver staining for nucleolus organizer regions indicated that the number of functional nucleolus organizer regions in PC-3 was proportional to the number of acrocentric chromosomes.

CARE AND USE OF TUMOR LINES

The transplantable tumors are distributed as frozen vials of tumor tissues or cell suspension. Transplantable tumors as well as cell culture lines are shipped as frozen vials of tissues on dry ice. Each tumor shipment includes an information sheet showing, among other items, the proper tumor designation; Cryopreserved date, In vivo host, etc.

Requested tumors are shipped in two to three weeks after the receipt of all completed paperwork. Shipments leave the Repository no later than Wednesday in order to reach their destination on weekdays. Before the shipment leaves the Repository, the Recipient is notified by email or fax of the waybill number and carrier, to allow time for the recipient to notify the Repository or make arrangements for receipt of cells, in the event that they will not be available to receive the shipment. An invoice for payment will follow and payment is due upon receipt. When vials are received they should be cultured right away, expanded and frozen down.

SUBMISSION OF TUMORS FOR CRYOPRESERVATION

Investigators who have unique and novel experimental tumor lines and are desirous of submitting their tumors to the Repository for cryopreservation and storage should write a letter of intent to the Project Officer. Upon acceptance, the Project Officer will inform the investigator in writing, and instructions on the procedure of shipment of the tumor materials to the Repository will be given. Tumor tissues or cells (frozen or ambient) are preferred over tumor-bearing animals.

At the Repository, the tumor line(s) will be tested for viral and bacterial contamination. When proven "clean," the line(s) will be expanded, in vivo or in vitro as appropriate, for large batch cryopreservation. Viability and growth of frozen tumors will be evaluated. The tumors will be included in the Repository's inventory, and upon joint approval of the submitting investigator and the Project Officer; they will be made available for distribution to the scientific community.

MOUSE TUMORS FROM THE JACKSON LABORATORY: These tumors formerly were maintained and distributed by the Jackson Laboratory. The list of available tumors begins on page 42 of the DCTD Tumor Repository Catalog. They were Cryopreserved at EG&G Mason Research Institute and are distributed only as vials of frozen tumor tissue. The required host animals for carrying the JAX tumors in serial transplantation may be obtained from:

Animal Resources
The Jackson Laboratory
600 Main Street
Bar Harbor, NE 04609
USA

T: 800.422.MICE
207.288.5845
F: 207.288.6150

FACTORS OF TRANSPLANTATION LOGISTICS: Since this inventory is concerned with transplantable animal and human tumor systems, the following information must be taken into consideration when planning studies involving tissues to be removed from cryopreservation:

a. Tumors have characteristic lag times (the time lapses between tissue implantation and the first palpable growths), which vary from several days to several months with different tumor systems.
b. Tumors also have characteristic rates of growth which markedly influence host survival, and which may vary from weeks to months with different tumor systems.
c. The above two factors are significantly prolonged in the first, and sometimes the second, transplant generation's post-freeze and thaw.
d. Histologically more complex tumors require two or three transplant generations, after thawing, before they return to normal histology and growth characteristics.

FREEZING PROCEDURE

Solid tumors are frozen as 2 x 2 x 2 mm fragments suspended in a freezing medium. Ascites or tissue-cultured cells are frozen as single cell suspension at a concentration of $10^6 - 10^7$ cells per ml. The freezing medium consists of appropriate tissue culture growth medium plus 10% DMSO and 10% fetal bovine serum.

Aseptically harvested ascites tumors are diluted in the freezing medium at a concentration of $10^6 - 10^7$ cells per ml. One ml suspension is pipetted into each 2 ml vial (Nunc cryotube). The vials are screw-capped tightly and labeled with a Repository number. Tissue cultured cells are prepared in a similar manner. For solid tumors, the aseptically excised tumor tissue is cut into 2 x 2 x 2 mm fragments after freeing it of necrotic materials. The fragments are placed in vials containing I.5 ml of freezing medium.

The processed tumors are frozen initially in a controlled slow-rate freezing apparatus at the rate of 0.5°C per minute to –20°C and 1°C per minute to -80°C. The frozen vials are stored in specific locations in the liquid nitrogen freezers in the Repository after the controlled freezing cycle.

RECOMMENDED THAWING PROCEDURE

Frozen tumor cells or tissues received from the Repository should be kept frozen at -70°C or lower until ready for use. For prolonged storage (more than two days), liquid nitrogen freezers are recommended.

The vials in which the cell lines are stored are reliable; however, they are very susceptible to contamination if thawed in a contaminated water bath. Thawing should be rapid, i.e., within 60-90 seconds. Place the vial in a warm water bath at 37-40°C and agitate vigorously to thaw. Immerse the vial in 70% ethanol before uncapping.

TROCARS FOR SUBCUTANEOUS IMPLANT OF FRAGMENTS ARE AVAILABLE FROM:

Popper and Sons, Inc.	NAME: Cancer Implant Needle Sets	
300 Denton Avenue	CATALOG NO:	7927
New Hyde Park, New York 11040	DESCRIPTION:	13 G X 3¼"
Telephone: (516) 248-0300	BRAND:	Perfektum

B. **PREPARATION OF TUMOR BREI** – Tumor brei is implanted with a syringe and 19-gauge or smaller needle. It may be implanted subcutaneously, intramuscularly, or intracerebrally. The tumor is excised, freed of normal fascia and debrided of necrotic tissue as described below. The tumor is minced with scissors and pressed through a sterile, 40-gauge stainless steel mesh into a sterile beaker in an ice bath. Add sterile, balanced salt solution, usually in an amount sufficient to prepare a 5% suspension. If specific numbers of cells are to be implanted, a sample of this brei can be counted in a hemocytometer and the brei diluted with sterile balanced salt solution as required. The usual inoculum size is o.2 or 0.3 ml per animal. No more than 30 minutes should elapse from tumor removal to transplantation in all recipient animals.

C. **ASCITES TUMORS** – These are implanted in the peritoneal cavity. Sacrifice and
Disinfect the donor animal as described above. Pin to a dissecting board, ventral surface up. Remove skin from the abdominal area. Withdraw fluid with a sterile syringe by inserting the needle through the abdominal wall. Transfer the fluid from the syringe to a sterile glass container in an ice bath. A cell count may be made, using a hemocytometer. Dilution is made with sterile, balanced salt solution. The usual mouse inoculum is 10^5 cells in 0.1 ml. It is injected intraperitoneally with a 23-gauge needle. No more than 60 minutes should elapse from the time fluid is taken from the donor and stored in the ice bath, and the diluted fluid is implanted in all the recipient animals.

BACTERIAL AND VIRAL MONITORING OF CRYOPRESERVED TISSUES: In addition to testing all freeze-runs for bacterial contamination, Cryopreserved tissues are tested for viral contamination by the MAP test. The viruses tested for are as follows: pneumonia virus of mice (PVM), reo virus-type 3 (Reo 3), Theiler's virus, Murine encephalitis (GD VII), polyoma (Poly), Sendia virus (Send), ectromelia, mouse pox (Ectro), lactic dehydrogenase virus (LDH).

IN VITRO ESTABLISHED CELL LINES:

A. **QUALITY CONTROL AND CHARACTERIZATION** – The quality control and characterization procedures for the incorporation of new cell lines into the Tumor Bank are as follows: Upon receipt, each cell line is immediately transferred to fresh antibiotic free medium and cultured for one week, after which it is tested for mycoplasma (PPLO) contamination. Standard culture

procedures under aerobic and anaerobic conditions, as well as the orcein staining procedure of Fogh, are used. The PPLO medium is extremely rich, and this procedure will also detect most bacterial and fungal contaminants.

B. **FREEZING AND STORAGE** – The cell cultures are frozen in ampules containing 1.0 ml of cell suspension at 2-6 x 10^6 cells/ml in fresh culture medium containing 10% DMSO. Freezing is performed as on page 2. Twenty-four hours after freezing, a representative ampule is removed, thawed, and viable cell count is performed, using the trypan blue dye exclusion procedure. The culture is also tested for its ability to initiate a heavy viable culture. Cell preparations, which show less than 50% viability or poor growth, are discarded and a new lot is prepared.

RECOMMENDED PROCEDURE FOR THAWING FROZEN CELL CULTURES

The vials in which the cell lines are stored are reliable; however, they are very susceptible to contamination if thawed in a contaminated water bath. The following procedures are provided to eliminate this problem.

Remove the ampule form the dry ice container and place it directly into a 37-40°C-water bath (or vessel) of freshly drawn water containing an effective concentration of disinfectant. The thawing should be vigorous and rapid (within 40-60 seconds). As soon as the thawing is complete, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point should be carried out under strict aseptic conditions in a sterile room, cubicle, or hood.

Transfer the contents of the ampule (1 ml volume) into a 100 mm petri dish or 25-cm2 flask containing 8-10 ml of the recommended culture medium, and incubate at the appropriate temperature and carbon dioxide level.

In order to remove the protective freezing additive (DMSO) from the culture medium, we suggest that the culture medium be changed 24 hours after thawing. If it is desired that the freezing additive be removed immediately or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid, and resuspend the cells in an appropriate volume of growth medium.

ViroMed Laboratories
A LabCorp Company

CHUNG cells.

Human Prostatic Carcinoma Cell Lines

Catalog Number	Cell Line Abbreviation	Product Description	Price
12-100	LNCaP	Direct parental prostate cancer cell line	$ 210.00
12-101	C4	Androgen dependent cell line, clone of LNCap	367.50
12-102	C4-2	Androgen independent cell line, clone of C4	367.50
12-103	C4-2B	Androgen independent cell line, clone of C4, developed in bone	367.50

A materials transfer agreement (MTA) is required prior to obtaining these cell lines.

Shipping Cost: Please contact your ViroMed account manager for specific information regarding domestic and international shipping costs.

For further information, please contact ViroMed Client Services at 800-582-0077.

Joanie → Janie.
procedure to obtain

www.Viromed.com

Revised February 19, 2009

ViroMed Laboratories
6101 Blue Circle Drive
Minnetonka, MN 55343-9108
Telephone: 800-582-0077
www.ViroMed.com

Chung Cancer Cell Lines – Procedure to Obtain

Submit the following information via email:

1. Chief Investigator's Name P. Foster
2. Intended usage statement
3. Exact shipping address (and billing, if different; we also accept Visa/MC for payment)

This information will then be forwarded and,

4. A Materials Transfer Agreement (MTA) will then be sent to you via email by our Sales Administration Dept. The above 3 items will be added to the MTA.

PLEASE NOTE: The terms of the MTA cannot be altered or changed. We can only accept the MTA as originally prepared. If terms of the MTA are altered, we will submit the changes to both our legal dept and MD Anderson Cancer Center for their approval. The approval process can take several weeks.

5. When the signed MTA (2 original copies) is received back, the ordering process can then proceed with verification of order and payment information.

For International shipments: Credit card or direct bank transfer is the preferred payment.

6. The order is then submitted to our production dept. We normally ship these cells out on a Monday or Tuesday; the orders may take up to several weeks to ship.

Reference Publications

1. LNCaP Human Prostrate Cancer Progression Model. Leland W.K. Chung, Ph.D. Urol Oncol 1996; 2:000-000
2. Androgen and retinoic acid interaction in LNCaP cells, effects on cell proliferation and expression of retinoic acid receptors and epidermal growth factor receptor. Ming-tang Li, Frank Richter, Chawnshang Chang, Robert J Irwin, Hosea FS Huang. BMC Cancer 2002 2:16.
3. Androgen-independent Cancer Progression and Bone Metastasis in the LNCaP Model of Human Prostrate Cancer. Cancer Research 54, 2577-2581, May 15, 1994.
4. Derivation of Androgen-Independent Human LNCaP Prostatic Cancer Cell Sublines: Role of Bone Stromal Cells. Int.J.Cancer 57, 406-412, 1994.

CELL-LINE SPECIFICATIONS

Name of Cell Line: C4-2

Origin: The human prostatic carcinoma cell line, *LNCaP* (1×10^6 cells; passage # 29) previously described [1] was co-inoculated into an athymic male nude mouse with (1×10^6) human fibroblasts derived from an osteosarcoma (cell line MS). The nude mouse host was castrated after 8 weeks incubation. A tumor specimen was excised after a total of 12 weeks. The *C4* cell line constitutes the *in vitro* cultured subline grown from the murine host's tumor [2]. When the *C4* sub-line was subsequently co-inoculated with MS osteosarcoma fibroblasts in a castrated athymic male nude mouse host for another 12 weeks by the same protocol described above. Prostatic epithelial cells cultured from the resultant tumor in this host constituted the *C4-2* subline[3].

Cell Line characteristics:
Morphology: fibroblast-like growth in tissue culture flasks; may form concentric zones of low cell growth upon reaching confluence.

Tumorigenicity & Osseous Metastasis: Orthotopic administration of 1×10^6 resuspended C4-2 cells in both intact and castrated athymic male nude mice yielded 100% tumorigenicity (20/20 and 14/14, respectively). Osseous prostate cancer metasteses were detected in both intact and castrated murine hosts (2/20 and 3/14, respectively) [3].

Soft-agar cloning: When 1×10^4 resuspended C4-2 cells were cloned on 0.3% soft agarose supplemented with 5% FBS and 2% TCM, colony formation was scored as 313 ± 15.3 relative to 12 ± 2.5 from the parental LNCaP cell line [2].

Androgen sensitivity: LNCaP sublines are androgen-independent.

Maintenance of C4-2, C4 and parental LNCaP cell lines:

Freezing Media: 10% DMSO + 90% T Media (containing 20% FBS)

Freezing Procedure: Freeze cells when cell layer becomes 90% confluent. Do not freeze at a confluency higher than 90%. Change media 1 day prior to freezing cells. Freeze at 1.5×10^6 cells per vial.

Frequency of Passage: Passage should be performed when confluency reaches 80-90%. If culture is allowed to remain too high of a confluency for more than a day or two, cells will begin to slough away from the monolayer. Over-grown passages can be difficult to re-plate as single cells. If cell number is too low when passing, cell growth tends to aggregate in isolated areas instead of growing and spreading across the flask.

Passing procedure: Cells are washed in PBS, pH 7.2. These cells are passed using a 0.05% trypsin/0.53mM EDTA solution. Apply trypsin/EDTA in the following volumes:

1.5 – 2ml/T25 flask
4 – 5 ml/T75 flask
5 – 6 ml/T175 flask

Leave trypsin/EDTA solution on cells for about 5 minutes, checking them frequently. Neutralize trypsinized cells with T media containing 5% FBS. Always centrifuge the trypsin/EDTA out of the culture and transfer cells to a new flask. Media volumes are as follows:

5ml media/T25 flask
15ml media/T75 flask
30ml media/175T flask

[1] Horoszewiez, JS et al. *Cancer Research* 43:1809-1818, 1983.

[2] Wu et al. Derivation of androgen-independent human LNCaP prostatic cell sublines: Role of bone stromal cells. *International Journal of Cancer* 57:406-412, 1994.

Final concentration in 500 ml: 0.244 ug/ml

5. ADENIN

Vendor: Sigma
Cat #: A-3159
Package size: 5 g

Aliquot concentration at 500x: 6.25 mg/ml in 0.1% BSA/PBS
Final concentration in 500 ml: 12.5 ug/ml

6. Penicillin and Streptomycin

Vendor: Mediatech
Cat #: 30-002-CI
Package size: 100 ml

Concentration:

Pen	50,000 units/ml
Strep	10,000 g/ml

Final Concentration:

Pen	100 units/ml
Strep	100 g/ml

7. TRYPSIN-EDTA (10X)
Ready to use

Vendor: Fisher
Cat #: BW17-161E
Package size: 100ml

Media Preparation:
400 ml DMEM
100 ml Ham's F12
50 ml Heat inactivated FBS (final conc. 10%)
5 ml 10X Pen/Strep (final conc. 1%)
1 ml of above supplements

Mix well and use, media will be good for 30 days.

Materials for trypsinizing the cells:
10X Trypsin (Fisher Cat # BW17-161E)
1X PBS

Procedure:

1. Thaw frozen cells quickly at 37° water bath and transfer into 15 ml tissue culture tube with 9 ml media.
2. Centrifuge at 12 RPM for 5 minutes at room temp or 4°
3. Aspirate media and wash cells with 1X PBS and centrifuge same as before.
4. Aspirate PBS and resuspend in 2 ml media and transfer to tissue culture plate or flask. Incubate at 37° with 5% CO2. **Do not disturb cells for at least four days. These cells are very sensitive and they may not adhere if they are disturbed.**

Splitting cells:

Warm up all reagents to 37° before use.

1. Split cells 1:4 when they are about 75-85% confluent by trypsinizing them with 21 ml 1x trypsin-EDTA for 5 min. at most at 37° (1ml for 100 mm plates and 25 cm2 tissue culture flask, 2 ml for 75 cm^2 flask and 3.5 ml for 150 cm^2 flask).
2. Stop trypsin with 9 ml of media; transfer cells into tissue culture tube and spin for 5 min at 1200 RPM at Room temp.
3. Aspirate media and wash cells with 10 ml 1X PBS, Centrifuge same as before and aspirate PBS.
4. Resuspend the pellet in 10 ml media and transfer into T75 tissue culture flask and incubate at 37° with 5% CO2.

Do not disturb the flask for at least two days.

MAINTENANCE OF C4, C4-2, C4-2B AND LNCaP CELL LINES:

Freezing Media: 10% DMSO + 90% T Media (containing 20% FBS)

Freezing Procedure: Freeze cells when layer becomes 90% confluent. Do not freeze at confluency higher than 90%. Change media 1 day prior to freezing cells. Freeze at 1-1.5x10^6 cells per vial.

Frequency of Passage: Passage should be performed when confluency reaches 80-90%. If culture is allowed to remain at too high of a confluency for more than a day or two, cells will begin to slough away from the monolayer. Over-grown passages can be difficult to re-plate as single cells. If cell number is too low when passing, cell growth tends to aggregate in isolated areas instead of growing and spreading across the flask.

Passing procedure: Cells are washed in PBS, pH 7.2. These cells are passed using a 0.05% trypsin/0,53mM EDTA solution. Apply trypsin/EDTA in the following volumes:

1.5 ml / T25 flask
4.0 ml / T 75 flask
5-6 ml / T 175 flask

Leave trypsin/EDTA solution on cells for about 5 minutes, checking them frequently. Neutralize trypsinized cells with T media containing 5% FBS. Always centrifuge the trypsin/EDTA out of the culture and transfer cells to a new flask. The lowest dependable amount of cells that will yield an even spread is 30,000 cells/ml – this number will vary depending each technician's own ability. Media volumes are as follows:

5 ml / T25 flask
15 ml / T75 flask
30 ml/T175 flask

Source: Cheryl Savola, PhD, ViroMed/LabCorp 10/14/04 – this document is the original information provided from UroCor/Dianon to ViroMed .

MDA-MB-231-luc-D3H2LN
Cell Line Information Sheet

General Information:

Designation:	MDA-MB-231-luc-D3H2LN
Tissue:	Human: adenocarcinoma; mammary gland; pleural effusion
Source of Parental Line:	ATCC (#HTB-26)
Derivation:	Harvested from metastatic lymph node tissue resulting spontaneously from an orthotopic MB-231-luc-D3H1 tumor after 12 weeks of in vivo growth.
Pathogen Testing:	Mouse Pathogen Test: IMPACT Profile I (PCR) at the University of Missouri Research Animal Diagnostic and Investigative Laboratory MDA-MB-231 Parental (passage 4): Negative 1/24/00 MDA-MB-231-luc-D3H2LN (passage 8): Negative 2/8/2008

Cotransfection:

Plasmids:	1) pGL3 control red (SV40-luc) (originally from Promega, construct from Chris Contag Lab at Stanford) 2) pSV40/Zeo (Invitrogen)
Transfection Method:	Lipofectamine/Plus Reagent (Invitrogen)

In Vitro Growth:

Recommended Media:	Eagle's MEM (500 ml) ATCC Cat. No.30-2003 Supplement the above with: Final concentration 50 ml FBS (Hyclone) 10%
Cell Doubling Time:	Approximately 24 hours (similar to parental cell line)
Split Ratio:	1:5 to 1:10, every 2-3 days using 0.25% Trypsin with EDTA
Zeocin Sensitivity:	50-100 µg/ml (If culturing cells >2 months, add 75ug/ml Zeocin in growth media to maintain optimum bioluminescence)

In Vitro Bioluminescence:

Lower Limit of Detection:	Able to detect ~20 cells in 200µl using IVIS® system when imaged at 1 min, 10 bin, level B/FOV 15 Bioluminescence: 189-208 photons/second/cell
Stability Over Time:	Signals stable for at least 2 months in culture without Zeocin selection; Signal drops by ~40% after 2 months

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ATCC® Number:	**HTB-26™** Order this Item	**Price:**	**$244.00**
Designations:	MDA-MB-231	**Depositors:**	R Cailleau
Biosafety Level:	1	**Shipped:**	frozen
Medium & Serum:	See Propagation	**Growth Properties:**	adherent
Organism:	*Homo sapiens* (human)	**Morphology:**	epithelial

Source: **Organ:** mammary gland; breast
Disease: adenocarcinoma
Derived from metastatic site: pleural effusion
Cell Type: epithelial

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

Receptors: epidermal growth factor (EGF), expressed
transforming growth factor alpha (TGF alpha), expressed

Tumorigenic: Yes

DNA Profile (STR): Amelogenin: X
CSF1PO: 12,13
D13S317: 13
D16S539: 12
D5S818: 12
D7S820: 8,9
THO1: 7,9.3
TPOX: 8,9
vWA: 15,18

Cytogenetic Analysis: The cell line is aneuploid female (modal number = 64; range = 57 to 68), with chromosome counts in the near-triploid range. Normal chromosomes N8 and N15 were absent. Clonal stable rearranged marker chromosomes are noted as well as unassignable chromosomes in addition to the majority of autosomes that are trisomic. Many of the marker chromosomes are identical to those shown in the karyotype reported by K.L. Satya-Prakash, et al.

Isoenzymes: AK-1, 1
ES-D, 1
G6PD, B
GLO-I, 2
Me-2, 1-2
PGM1, 1-2
PGM3, 1

Age: 51 years adult

Berkeley Lab

« MDAMB415 MDAMB435S »

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National Laboratory
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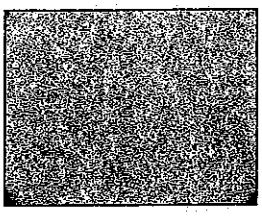
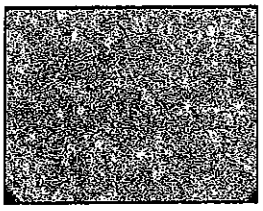
Berkeley, CA 94710-2722

MDAMB435

ATCC	
Specimen Origin	
Source	Georgetown
Organism	Homo Sapiens (Human)
Tissue	Skin
Tumourigenic	
Morphology	Epithelial
Age Status	
Ethnicity	
Gender	Female
Cytogenetic Analysis	
Growth Properties	Adherent
Doubling Time	
Culture Media	DMEM, 10%FBS
Split Ratio	
Freeze Media	culture medium 95%; DMSO, 5%
Comments	There is debate regarding whether this is a breast cancer cell lines or not, since it clustered with melanoma samples in PMID: 12354931, 15679052, 15150101. A recent article by Rae et al (PMID 17004106) definitively identifies this as a line derived from the M14 melanoma cell line. For more information see: http://dtp.nci.nih.gov/docs/misc/common_files/mda-mb-435-update.html
References	
Mutations	



10X



20X

Synonymous Cell Lines

MDAMB435S

MDA-MB-435 is a Melanoma cell line, not a breast cancer cell line

MDA-MB-435, a member of the NCI-DTP panel of 60 human tumor cell lines, has been used for decades as a model of metastatic human breast cancer. This cell line was derived at M.D. Anderson in 1976 from a pleural effusion from a 31-year old woman with a history of breast cancer (Cailleau R, Olive M, Cruciger QV. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. In Vitro. 1978 Nov;14(11):911-5. ; Brinkley BR, Beall PT, Wible LJ, Mace ML, Turner DS, Cailleau RM. Variations in cell form and cytoskeleton in human breast carcinoma cells in vitro. Cancer Res. 1980 Sep;40(9):3118-29.) Further background information on this cell line may be found at the M.D. Anderson Breast Cancer Cell Line Database.

Recent advances in gene expression analysis allow the opportunity to more fully characterize tumor cell lines. Analysis of MDA-MB-435, in conjunction with the rest of the NCI60 panel, revealed that the pattern of gene expression for MDA-MB-435 more closely resembled that of melanoma cell lines than of other breast tumor lines (Ross et al. Systematic variation in gene expression patterns in human cancer cell lines. Nat Genet 2000 Mar;24(3):227-3.)

These findings prompted Ellison et al. to undertake a more detailed study of the characteristics of MDA-MB-435 (Ellison G, Klinowska T, Westwood RF, Docter E, French T, Fox JC. Further evidence to support the melanocytic origin of MDA-MB-435. Mol Pathol. 2002 Oct;55(5):294-9.). They measured expression of several breast-specific genes and several melanoma-specific genes in MDA-MB-435 (obtained from the American Type Culture Collection), as well as in other breast tumor cell lines, melanoma cell lines and normal breast. Breast-specific genes were not detectably expressed in MDA-MB-435 or in the melanoma lines, but were detected in most of the breast tumor cell lines as well as normal breast. However, melanocyte-specific genes were expressed in MDA-MB-435, as well as in most of the melanoma lines, but were not detectable in the other breast tumor cell lines. Additionally, xenografts of MDA-MB-435 implanted into mammary fat pads of female SCID mice showed immunohistochemical staining consistent with melanocytic origin.

More recently single nucleotide polymorphism (SNP) array analysis revealed that MDA-MB-435 is derived from the same individual as the melanoma cell line M14 (Garraway LA, et al. Integrative genomic analyses identify MITF as a lineage survival oncogene amplified in malignant melanoma. Nature. 2005 Jul 7;436(7047):117-22. ; http://www.sanger.ac.uk/genetics/CGP/NCI60/).

The NCI Developmental Therapeutics Program obtained MDA-MB-435 from Dr. Patricia Steeg (NCI) -- Dr. Steeg obtained the line from M.D. Anderson. The DTP has obtained DNA fingerprinting analysis of the MDA-MB-435 in the DTP repository, as well as MDA-MB-435 from the ATCC (which obtained their sample from M.D. Anderson). DNA fingerprinting on all MDA-MB-435 samples are consistent with their derivation from the same individual. Thus the mix-up with the melanoma cell line M14 likely happened early in the history of the cell line.

Note added 8/5/2009: The panel designation for this cell line continues to be a topic for discussion, as seen in a recent publication by Chambers.
(MDA-MB-435 and M14 cell lines: identical but not M14 melanoma? Cancer Res. 2009 Jul 1;69(13):5292-3.)

Note added 8/2007: A recent publication by Rae et al. used karyotype, CGH, and microsatalite polymorphism analyses, combined with bioinformatics analysis of gene expression and SNP data and concluded that "All currently available stocks of MDA-MB-435 cells are derived from the M14 melanoma cell line". (Rae JM et al. MDA-MB-435 cells are derived from M14 Melanoma cells--a loss for breast cancer, but a boon for melanoma research. Breast Cancer Res Treat. 2007 Jul;104(1):13-9.)

Health Science Research Resources Bank
Japan Health Sciences Foundation

http://www.jhsf.or.jp/cgi-bin/HSRRB/C_ViewDetail.cgi?jcrb=JCRB0156

JCRB No.	JCRB0156
Cell Name	KHYG-1
Profile	Human natural killer cell line with a p53 point mutation as a model for p53-associated leukemogenesis and a model for differentiation of NK/T cells.
Animal	human
Species	Homo sapiens
Sex	F
Age	45-year-old
Tissue	peripheral blood
Case History	aggressive NK cell leukemia
Metastasis	
Genetics	p53 point mutation and cytogenetic characteristics.
Lifespan	infinite
Morphology	lymphocyte-like
Characteristics	high NK/LAK activity, IFN gamma production etc.
Classification	tumor
Establisher	Yagita,M.
Depositor	Yagita,M.
Medium	RPMI1640 medium with 10% fetal calf serum and 100 units/ml of rIL-2(Shionogi).
Passage Method	Simple dilution because of suspension cell culture. Subculture every 3-4 days with split ratio = 1/4.
Passage Cell No.	Split 1/4 every 3-4 days.

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Product Description

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ATCC® Number: CRL-11147™ Order this Item **Price:** $329.00

Designations:	A2058
Depositors:	W Stetler-Stevenson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** skin **Disease:** melanoma **Derived from metastatic site:** lymph node
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.
Applications:	transfection host (technology from amaxa)
Receptors:	nerve growth factor (NGF), expressed laminin, expressed
Tumorigenic:	Yes
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 13,14 D16S539: 9,13 D5S818: 9,12 D7S820: 11 THO1: 7,9 TPOX: 8 vWA: 14,18
Age:	43 years adult
Gender:	male
Ethnicity:	Caucasian
Comments:	This cell line is highly invasive and provides a source of cellular invasion associated proteins (such as the 72000 dalton type IV collagenase. Tissue inhibitor of metalloproteinase-2 [TIMP-2], autocrine motility factor and the 67000 dalton laminin receptor.

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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 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 1:6 to 1:12 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 30-2002
recommended serum:ATCC 30-2020

References:

22590: Fabricant RN, et al. Nerve growth factor receptors on human melanoma cells in culture. Proc. Natl. Acad. Sci. USA 74: 565-569, 1977. PubMed: 265522
23263: Sherwin SA, et al. Human melanoma cells have both nerve growth factor and nerve growth factor-specific receptors on their cell surfaces. Proc. Natl. Acad. Sci. USA 76: 1288-1292, 1979. PubMed: 375235
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23404: Stetler-Stevenson WG, et al. The activation of human type IV collagenase proenzyme. Sequence identification of the major conversion product following organomercurial activation. J. Biol. Chem. 264: 1353-1356, 1989. PubMed: 2536363
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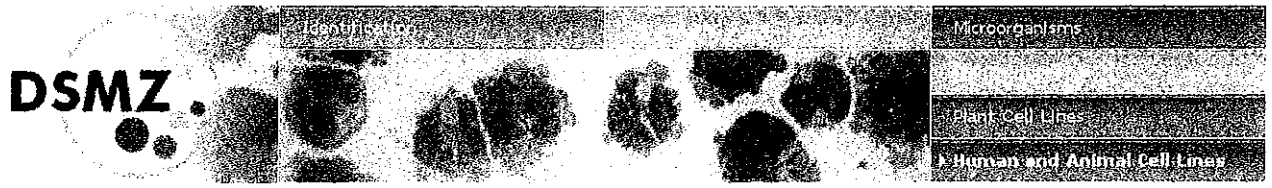

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EM-2

Cell line:	EM-2
Cell type:	human chronic myeloid leukemia in blast crisis
DSMZ no.:	ACC 135
Origin:	established from the bone marrow of a 5-year-old Caucasian girl with Philadelphia-chromosome positive chronic myeloid leukemia (CML) in second relapse in 1980 (after bone marrow transplantation day +28); sister cell line of EM-3 (DSM ACC 134)
References:	Raskind et al., Cancer Genet Cytogenet 25: 271-284 (1987), PubMed ID 2 target=_blank>3030532 Keating, Baillieres Clin Haematol 1: 1021-1029 (1987), PubMed ID 2852 target=_blank>3332852 review: Drexler et al., Leuk Res 24: 109-115 (2000), PubMed ID 10654445
Depositor:	Dr. A. Keating, Toronto General Hospital, Ontario, Canada

DSMZ Cell Culture Data

Morphology:	single, round to oval cells in suspension
Medium:	90% RPMI 1640 + 10% FBS
Subculture:	maintain at 0.2-1.0 x 10^6 cells/ml; split ratio of 1:2 every 2-3 days; seed out at ca. 0.3-0.5 x 10^6 cells/ml
Incubation:	at 37 °C with 5% CO_2
Doubling time:	ca. 35-40 h
Harvest:	maximum density at about 1.0-1.5 x 10^6 cells/ml
Storage:	frozen with 70% medium, 20% FBS, 10% DMSO at about 6 x 10^6 cells/ampoule

DSMZ Scientific Data

Mycoplasma:	negative in DAPI, microbiological culture, RNA hybridization, PCR assays
Immunology:	CD3 -, CD4 +, CD13 +, CD14 -, CD15 +, CD19 -, CD33 +, CD34 -, HLA-DR -; image
Fingerprint:	same DNA profile as EM-3 using multiplex PCR of minisatellite markers
Species:	confirmed as human with IEF of AST, MDH, NP
Cytogenetics:	human hypertriploid karyotype with hypotetraploid sideline - 74(70-86)<3n>X, -X, -X, +3, +4, +6, +6, +6, +8, -9, +11, -14, -14, +15, +17, -19, +21, +22, +mar - der(5)t(5;?)(q13-15;?), der(9)t(9;22)(q34;q11), i(17q)x2 - in some cells up to three copies of der(9) are present - the karyotype overlaps that of EM-3 (DSM ACC 134) and corresponds with those reported for both EM-2 and EM-3
Molec. Genetics:	
Viruses:	ELISA: reverse transcriptase negative; PCR: EBV -, HBV -, HCV -, HHV-8 -, HIV -, HTLV-I/II -, SMRV -

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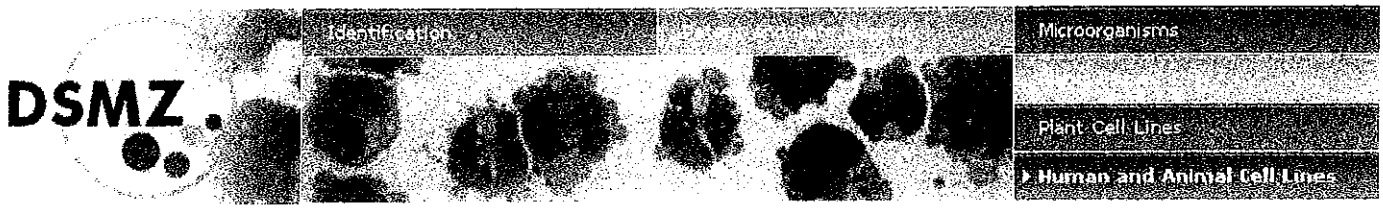

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MEC-1

Cell line:	MEC-1
Cell type:	human chronic B cell leukemia
DSMZ no.:	ACC 497
Origin:	established in 1993 from the peripheral blood of a 61-year-old Caucasian man with chronic B cell leukemia (B-CLL in prolymphocytoid transformation to B-PLL); serial sister cell line of MEC-2 (DSM ACC 500)
References:	Stacchini et al., Leuk Res 23: 127-136 (1999), PubMed ID 10071128
Depositor:	Prof. F. Caligaris-Cappio, Hospital Mauriziano Umberto, Turin, Italy

DSMZ Cell Culture Data

Morphology:	round to polymorphic cells growing in suspension, singly or partly in small aggregates, a few cells are slightly adherent
Medium:	90% Iscove's MDM + 10% FBS
Subculture:	split saturated culture 1:2 to 1:3 every 2-3 days; seed out at ca. 0.5 x 10^6 cells/ml; after thawing, during the first week cells should be kept with 20% FBS initially; culture in 24-well-plate is of advantage; maintain at about 0.5-2.0 x 10^6 cells/ml
Incubation:	at 37 °C with 5% CO_2
Doubling time:	ca. 40 hours
Harvest:	cell harvest of about 2.0 x 10^6 cells/ml
Storage:	frozen with 70% medium, 20% FBS, 10% DMSO at about 5 x 10^6 cells/ampoule

DSMZ Scientific Data

Mycoplasma:	contamination was eliminated with BM-Cyclin (tiamulin & minocycline), then negative in microbiological culture, RNA hybridization, PCR assays
Immunology:	CD3 -, CD10 -, CD13 -, CD19 +, CD20 +, CD34 -, CD37 +, cyCD79a +, CD80 +, CD138 -, HLA-DR +, sm/cyIgG -, sm/cyIgM +, sm/cykappa +, sm/cylambda -
Fingerprint:	same DNA profile as MEC-2 using multiplex PCR of minisatellite markers
Species:	confirmed as human by cytogenetics
Cytogenetics:	human near-diploid karyotype with 10% polyploidy - 46(44-47)<2n>XY, -2, +7, -12, +1-2mar, t(1;6)(q22-23;p21), add(7)(q11), der(10)(10pter->q22::?::2q11->qter), del(17)(p11) - small acf/mar present in most cells - resembles published karyotype
Molec. Genetics:	
Viruses:	PCR: EBV +, HBV -, HCV -, HIV -, HTLV-I/II -, SMRV -

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ATCC® Number:	**CCL-86™**	Order this Item	Price:	**$272.00**

Designations:	Raji
Depositors:	W Henle
Biosafety Level:	2 [CELLS CONTAIN HERPESVIRUS (EBV)]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	suspension
Organism:	*Homo sapiens* (human)
Morphology:	lymphoblast
Source:	**Disease:** Burkitt's lymphoma **Cell Type:** B lymphocyte;
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.
Isolation:	**Isolation date:** 1963
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Resistance:	The cells are partially resistant to poliovirus and vesicular stomatitis viruses.
Reverse Transcript:	negative
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 10,12 D13S317: 13 D16S539: 8,11 D5S818: 10,13 D7S820: 10 THO1: 6,7 TPOX: 8,13 vWA: 16,19
Cytogenetic Analysis:	Karyotype 100% stable within male diploid stemline of 46. Cells with 47 chromosomes frequently contained an extra "E" group chromosome.; There is 6% polyploidy and occasional disparity in the size of the homologs of the number 1 chromosome and the number 4 chromosome.
Age:	11 years

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22572: Moore PS, et al. Primary characterization of a herpesvirus agent associated with Kaposi's sarcoma. J. Virol. 70: 549-558, 1996. PubMed: 8523568
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Isoenzymes:	AK-1, 1 ES-D, 1 G6PD, B GLO-I, 2 Me-2, 0 PGM1, 0 PGM3, 1
Age:	53 years
Gender:	female
Comments:	The continuous cell line K-562 was established by Lozzio and Lozzio from the pleural effusion of a 53-year-old female with chronic myelogenous leukemia in terminal blast crises. [22609] The cell population has been characterized as highly undifferentiated and of the granulocytic series. [26059] Studies conducted by Anderson, et al., on the surface membrane properties led to the conclusion that the K-562 was a human erythroleukemia line. [26060] The K-562 cell line has attained widespread use as a highly sensitive in vitro target for the natural killer assay. [1101] [48829] [48830] See Pross, et al. for a detailed analysis of the in vitro assay of NK cells including the mathematics of quantitation of NK cell activity. [48833] K-562 blasts are multipotential, hematopoietic malignant cells that spontaneously differentiate into recognizable progenitors of the erythrocytic, granulocytic and monocytic series. [26061] The effect of inducers on sublines derived from the original K-562 cell line have been reviewed by Koeffler and Golde. [867] Cultures from the ATCCstock have been shown to exhibit this sensitivity for assessing human natural killer activity. Karyological studies on various K-562 sublines have been classified into three groups (A,B,C) by Dimery, et al. [26063] The strain obtained by the ATCCmost closely resembles the B population. Occurrence of the Philadelphia chromosome, however, was of much lower frequency; none detected in 15 metaphases examined. The line is EBNA negative.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Iscove's Modified Dulbecco's Medium, Catalog No. 30-2005. 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

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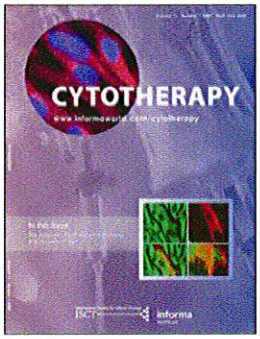
HMSC



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Improved isolation protocol for equine cord blood-derived mesenchymal stromal cells

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Improved isolation protocol for equine cord blood-derived mesenchymal stromal cells

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Background aims

A robust methodology for the isolation of cord blood-derived multipotent mesenchymal stromal cells (CB-MSCs) from fresh umbilical cord blood has not been reported in any species. The objective of this study was to improve the isolation procedure for equine CB-MSCs.

Methods

Pre-culture separation of red and white blood cells was done using either PrepaCyte®-EQ medium or Ficoll-Paque™ PREMIUM density medium. Regular FBS and MSC-qualified FBS were compared for their ability to support the establishment of putative primary MSC colonies.

Results and conclusions

Our results indicate that PrepaCyte®-EQ medium is superior to Ficoll-Paque™ PREMIUM density medium for the isolation of putative equine CB MSC and that MSC-qualified FBS may improve the isolation efficiency.

Keywords

Cord blood, horse, isolation, mesenchymal stromal cells.

Introduction

Consistent and reproducible isolation of cord blood (CB)-derived multipotent mesenchymal stromal cells (MSC) from fresh umbilical cord blood has not been reported in any study regardless of the species concerned. The highest reported isolation percentage has been 63% on selected cord blood samples. Developing a robust isolation method for these progenitor cells is crucial for the widespread acceptance of cord blood as a cell source for autologous use later in life. The objective of this study was to improve the isolation procedure for equine CB MSC by evaluating different cell separation methods and fetal bovine serum (FBS) batches. Our results indicate that PrepaCyte®-EQ medium is superior to Ficoll–Paque™ PREMIUM density medium for the isolation of putative equine CB MSC and that MSC-qualified FBS may improve the isolation efficiency.

Methods

Study design and cell line establishment

Each of five fresh CB samples was split into three groups of 42 mL, from which the nuclear or mononuclear cell (MNC) fraction was isolated by one of three methods: PrepaCyte®-EQ medium (PEQ; BioE Inc., St Paul, MN, USA), Ficoll-Paque™ PREMIUM medium (1.077 g/mL; GE Healthcare, Mississauga, ON, Canada) loaded with undiluted whole blood (FUD) and Ficoll-Paque™ PREMIUM medium loaded with diluted whole blood (FD). The manufacturer's guidelines were followed using A and B salt solutions (Figure 1). The cell suspension from each of the three separation methods was split into two groups to allow assessment of two different batches of FBS, one being regular FBS and one being 'MSC-qualified' FBS.

The CB was collected as described previously [1]. The PEQ protocol was based on the manufacturer's guidelines using 7 mL whole blood mixed with 7 mL PEQ in 15-mL tubes. In the FUD and FD groups, 7 mL sample were loaded on to 6 mL Ficoll-Paque PREMIUM density medium (1.077 g/mL) in 15-mL tubes (six tubes total) prior to cell separation, as per the manufacture's guidelines. The nucleated cells in each culture group were suspended in 10 mL isolation medium (low-glucose Dulbecco's modified Eagle medium; DMEM; Lonza, Wakersville, MD, USA), 30% regular FBS (Invitrogen, Burlington, ON, Canada) or

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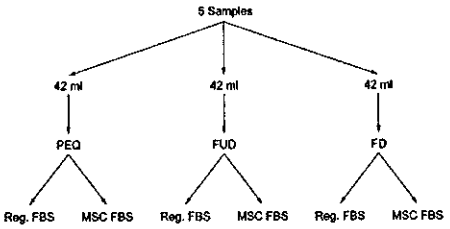

Figure 1. Study design. Each of five fresh CB samples was split into three groups of 42 mL and the nuclear or MNC fraction was isolated using the PEQ, FUD or FD protocol. Cell suspensions from each of the three separation groups were split further into two groups to allow assessment of two different batches of FBS, regular FBS (Reg. FBS) and MSC-qualified FBS (MSC FBS).

MSC-qualified FBS (Invitrogen), with low dexamethasone (10^{-7} M; Sigma, Oakville, ON, Canada), penicillin (100 IU/mL; Invitrogen), streptomycin (0.1 mg/mL; Invitrogen) and L-glutamine (2 mM; Sigma). One milliliter was submitted for automated differential cell counting. As most reported seeding densities are based on manual cell counts, which do not discriminate between granulocytes and monocytes, seeding densities were based on the total white blood cell count. Cells were seeded in six-well polystyrene plastic culture plates and incubated at 38.5°C in humidified atmosphere containing 5% CO_2 in air.

Population doubling time (PDT) was calculated from passage (P) 2 onwards as follows: PDT = (log(number of cells obtained at subculture per cm^2/[cell seeding density per cm^2)/log 2)/d], where d is the number of days in culture.

Trilineage differentiation studies

Three cryopreserved cell lines from the PEQ group were assessed for adipogenic, chondrogenic and osteogenic potency, as demonstrated previously for cells derived using Ficoll medium separation [1].

For adipogenesis, each cell line was cultured for 10 days in either continuous expansion culture medium (regular FBS), as defined above, or continuous adipogenic induction medium (BulletKit®; Lonza). The induction medium consisted of 1 µM dexamethasone, 0.5 mM 3-isobutyl-1-methyl-xanthine (IBMX), 10 µg/mL recombinant human (rh) insulin, 0.2 mM indomethacin and 10% fetal calf serum (FCS) in DMEM. The fetal calf serum included the commercial medium kit for adipogenic induction was discarded and substituted with 15% rabbit serum (Sigma). Oil Red O staining was done as described previously except rinsing and staining volumes of 200 µL were used because of the smaller well size [1]. The AdipoRed™ assay (Lonza) was used according to the manufacturer's protocol for 96-well plates with the exception of a 520-nm emission filter instead of 572 nm or 535 nm. The suitability of the 520-nm emission filter was discussed with the manufacturer's scientific product adviser prior to use. The total protein content of the AdipoRed assayed wells was determined as described previously using a DC Bio-Rad protein method (DC protein assay reagents package; Biorad, Mississauga, ON, Canada) [1].

Chondrogenic differentiation was performed using a micromass culture system [1,2]. Undifferentiated cells (2.5×10^5 cells) were cultured for 2 and 4 weeks in 0.5 mL complete chondrogenic differentiation medium (Lonza) containing 10 ng/mL transforming growth factor-beta 3 (TGF-β3). Pellets were fixed in 10% formalin, imbedded in paraffin blocks and sectioned into 5-µm sections. Hematoxylin and eosin (Sigma) as well as Safranin O (Sigma) and Alcian Blue (Sigma) staining was used to evaluate chondrogenic differentiation histologically.

Osteogenesis was induced using osteogenic induction medium (100 nm dexamethasone and 10 mM β-glycerophosphate; Sigma), 0.05 mM L-ascorbic acid-2-phosphate (Fluka Biochemika, Sigma) and 10% FBS in low-glucose DMEM (Lonza) for 10 days and compared with control cultures exposed to regular expansion medium. Osteogenic differentiation was evaluated qualitatively using Alizarin Red S staining and semi-quantitatively by alkaline phosphatase, calcium and protein assays, as reported elsewhere [1].

Statistical analysis

Data were analyzed using ANOVA, Box Cox transforms, linear modeling and contrast methods as applicable.

Results and Discussion

Significantly more MNC were isolated in the PEQ group compared with the FUD and FD groups (Figure 2A). The average total numbers of MNC seeded from each of the processed 42 mL cord blood within each group were as follows: PEQ, $4.5.0 \times 10^8$ cells (range 2.43×10^8 – 8.91×10^8); FUD, 2.88×10^7 cells (range 9.0×10^6 – 5.4×10^7); FD, 2.52×10^7 cells (range 9.0×10^6 – 5.4×10). Significantly more colonies were established in the PEQ group

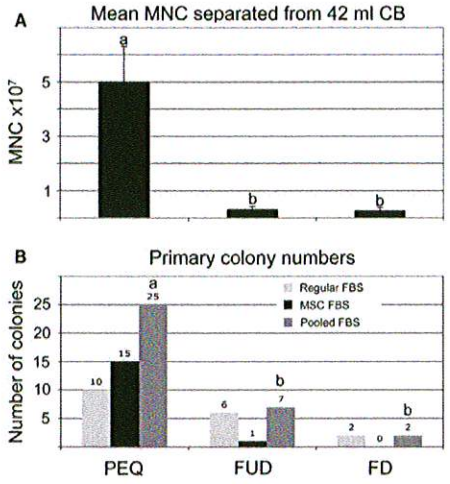

Figure 2. MNC concentrations and primary colony numbers. (A) Use of PEQ yielded significantly higher concentrations of MNC than methods of undiluted and diluted blood loaded onto Ficoll-Paque PREMIUM (FUD and FD groups, respectively). (B) Significantly more colonies were noted in the PEQ group than with either FUD or FD groups. Solid light gray column, regular FBS; solid black column, MSC-qualified FBS; gray checked column, regular and MSC-qualified FBS combined. Statistical significant differences at the level of $P < 0.05$ *are indicated by different lower case letters.*

compared with the FUD and FD groups (Figure 2B). In the PEQ group, 25 colonies were noted, with 15 cultured in MSC-qualified FBS and 10 cultured in regular FBS. There were no significant differences in the number of colonies established in the FUD and FD groups of seven and two colonies, respectively. In the FUD and FD groups only one colony was noted in the MSC-qualified FBS culture group. The difference in colony numbers using different serum sources was insufficient for statistical comparisons. The importance of screening multiple FBS batches to determine which ones provide the best support for isolation of human bone marrow-derived MSC has been reported previously [3,4]. Further work is required to determine whether commercially available MSC-qualified FBS improves the isolation of equine MSC.

After combining the two serum groups, the isolation success of putative MSC per blood sample for each of the three different MNC separation methods was as follows: PEQ, 100% (5/5); FUD, 60% (3/5); FD, 20% (1/5). A 100% isolation success for putative CB MSC, as noted in the PEQ group, is remarkable. In a previous study, a protocol similar to the FUD group of this study was used. Interestingly, the isolation success in the FUD group of the current study was 60%, which was very similar to the 57% of the previous report [1]. Recent reports on human CB MSC isolation illustrate that consistent and reproducible isolation continues to be challenging, with less than 60% isolation success [5–7].

The overall PDT per day for 15 cell lines was 0.89 (SEM 0.1029). PDT for equine adipose tissue-derived MSCs (AT-MSCs) has been reported as 2:190.9 days for the first 10 population doublings [8]. The PDT for equine bone marrow-derived MSCs (BM-MSCs) has been reported as 1.490.22 days for passages after initial cell line establishment, and was associated with a prolonged PDT of 4.991.6 days [9]. The isolated CB-MSCs appears to be more proliferative than AT-MSCs and BM-MSCs, although different culture conditions complicate a direct comparison.

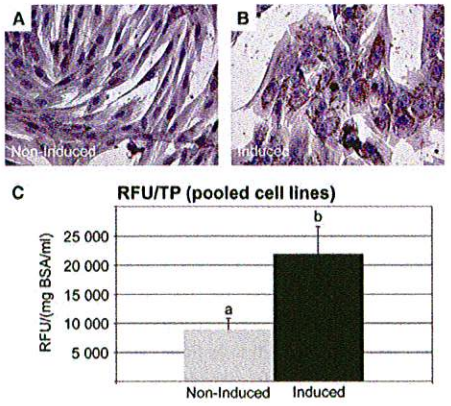

Figure 3. Assessment of adipogenesis. (A) Oil Red O staining of non-induced control cell cultures at day 10, magnification × 200. Few cells contain small Oil Red O-positive vacuoles. (B) Oil Red O staining of induced cell cultures on day 10, magnification × 200. Note the change of morphology toward large cuboidal cells and robust presence of Oil Red O vacuoles in all cells. The other two cell lines tested showed similar results. (C) Quantitative assessment of intracellular lipid content using the AdipoRed assay. The relative fluorescent unit (RFU) values were normalized against the total protein content of the culture wells. Induced cultures contained significantly more intracellular lipids than non-induced cultures ($P = 0.0006$).

All three tested cell lines could be induced to form pre-adipocytes and a statistically significant ($P = 0.0006$) increase in adipogenesis was measured in induced cultures compared with non-induced cultures using the quantitative AdipoRed assay (Figure 3). All three cell lines tested could also be induced toward the osteogenic cell lineage, as demonstrated by marked Alizarin Red S-positive calcium deposits in the induced cultures and a significant quantitative increase in alkaline phosphatase activity as well as calcium concentration in induced cultures compared with non-induced cultures (Figure 4). One in three tested cell lines showed robust chondrogenic differentiation, as demonstrated by an increase in pellet size over time as a result of production of extracellular matrix (ECM) and evidenced by the positive stain of glycosaminoglycans by Alcian Blue and Safranin O together with hyaline-like cartilage morphology of lacunae containing the chondrocyte (Figure 5). The reasons for the variable chondrogenic potential are undetermined. A variable chondrogenic potential of clonally expanded bovine BM MSC has been reported elsewhere [10]. A hierarchical loss of trilineage potency in human BM MSC has been advocated, with MSC losing adipogenic followed by chondrogenic and finally osteogenic potential loss [11]. In these differentiation experiments of human clonal BM MSC lines, cells with adipogenic and osteogenic potential, but not chondrogenic potential, were never identified [11]. More detailed investigation of chondrogenesis examining gene expression of chondrogenic markers could help determine whether the apparent chondrogenic response is truly absent.

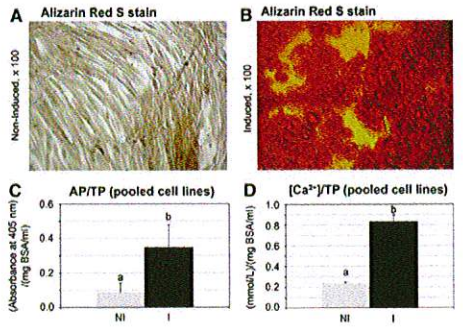

Figure 4. Assessment of osteogenesis. (A) Alizarin Red S (ARS) staining of non-induced control cell cultures at day 10, magnification × 100. (B) ARS staining of induced cell cultures at day 10, magnification × 100. Extensive ARS-positive ECM and no individual cells can be discerned. The other two cell lines tested showed similar results. (C) Quantitative assessment of alkaline phosphatase (AP) activity after 25 min of enzymatic activity. The AP absorbance values were normalized against the total protein (TP) content of the culture wells. Induced cultures (I) showed significantly more AP activity than non-induced cultures (NI) (P = 0.0012). (D) Quantitative assessment of calcium content per culture well. The calcium concentrations were normalized against the TP content measured in the AP wells. Induced cultures showed significantly more calcium deposits than non-induced cultures (P = 0.0006). Different letters above the bar graphs indicate statistically significant differences (P < 0.05).

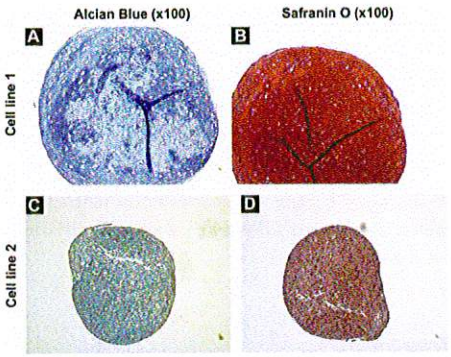

Figure 5. Assessment of chondrogenesis. One of three tested PEQ-derived putative MSC lines showed positive chondrogenesis (A, B). All images are at 100 times magnification with colors auto-adjusted using Adope® Photoshop® 7.0 software. Note the larger pellet size of (A) and (B) compared with the absent chondrogenesis of (C) and (D), reflecting deposition of ECM. Glycosaminoglycans, in the ECM, are positively stained with Alcian Blue and Safranin O. Chondrocytes within lacunae are also evident, consistent with a hyaline-like cartilage morphology. (C) and (D) are negative for the stains.

Acknowledgements

Daniel Gillis is acknowledged for statistical assistance. Windfield Farms, Oshawa, Ontario, Canada, is acknowledged for collection of cord blood.

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donated by BioE. BioE also markets similar products for human use: PrepaCyte®-CB and PrepaCyte®-WBC. PrepaCyte®-CB is a clinical product intended for processing human cord blood. PrepaCyte®-WBC, for research use only, is intended for processing human cord blood, peripheral blood or BM.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Application for permit to import human pathogen(s) / Demande de permis d'importation d'un (d')agent(s) anthropopathogène(s)

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1. Applicant - Name, address and postal code / Demandeur - Nom, adresse, et code postal

Gabrielle M. Siegers, Foster Lab, Robarts Research Institute, PO Box 5015, 100 Perth Dr. Rm 3296, London, ON, N6A 5K8

Facsimile / Télécopieur: 519-931-5224

Telephone no. / N° de téléphone: 519-663-5777 ext 24086

2. Supplier - Name and address / Fournisseur - Nom et adresse

Anna Kreutzman, Hematology Research Unit, Biomedicum Helsinki, C428b, Department of Medicine, Helsinki University Central Hospital, P.O. Box 700, FI-00029 HUCH, Finland

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

blood samples from human chronic myeloid leukemia patients in Finland

4. Mode of transportation / Mode de transport

courier (air/road)

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

various

6. Quantity of material to be imported and in the case of a human pathogen belonging to risk group 2, any intervals at which, or period during which, the pathogens are to be imported.
Quantité de la matière à importer - Dans le cas d'un agent anthropopathogène du groupe de risque 2, toute intervalle ou période d'importation.

Approximately 600 ml human blood (60-90 ml/shipment at 4-6 week intervals) will be imported in a one-year period.

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

Our laboratory meets Containment Level 2 requirements as per the Laboratory Biosafety Guidelines 3rd edition.

Additional information attached / Renseignements complémentaires ci-joint: ☐ Yes / Oui ☒ No / Non

8. Address of location where the human pathogen is to be used / Adresse du lieu où sera utilisé l'agent anthropopathogène

Robarts Research Institute,100 Perth Dr., London, ON, N6A 5K8

9. Method of treatment of material for the purposes of decontamination, sterilization and waste disposal
Méthode de traitement de la matière aux fins de décontamination, de stérilisation et de l'élimination des déchets

Liquid waste will be aspirated into a designated flask and treated with bleach prior to disposal.

10. Work objectives, proposed plan of work and additional pertinent information / Objectifs des travaux, plan de travail proposé et autres renseignements utiles

Gamma delta T cells (GDTc) constitute 2-5% of circulating lymphocytes in human blood. Since these cells elicit cytolytic responses against a variety of tumors in vitro and in vivo, immunotherapy using GDTc is currently under investigation in clinical trials. Using our GDTc isolation and expansion protocol we aim to: 1) assess whether GDTc can be expanded from the blood of chronic myeloid leukemia (CML) patients; 2) determine clonality of expanded GDTc; 3) assess functional capacity of expanded GDTc; 4) determine mechanisms of cytotoxicity of GDTc against CML cell lines and autologous tumours; and 5) assess iron oxide labeling of expanded GDTc for single cell imaging via MRI.

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

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

Containment level (Check one block only) / Niveau de confinement (Ne cocher qu'une seule case)

☐ 1 ☒ 2 ☐ 3 ☐ 4

Signature of applicant - Signature du demandeur: Gabrielle M Siegers

Date: 12 Jan 2011

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

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

HC/SC 4324 12-2004)

Canada

GDTc cloning protocol (P. Fisch)

Protocol:

1) Isolate PBMCs from 20 ml blood.
2) FACS sort (and count) γδ T cells by positive selection directly into vial containing irradiated feeder cells* (1 x 10^6/ml) in medium, then mix well with fresh medium. The laser should be turned down as low as possible <100 mW.
3) Plate out at 1 cell/well (according to FACS count) into 96 well plates. Actual density will likely be 0.5 cell/well. Grow at 7% CO_2. Clones should come up after 9-12 days.
4) Wait another 2-3 days, then pick the clones, transferring them from 1 well into 6 into fresh medium with fresh feeders. Use only the inner 60 wells, thus you have 10 clones/plate.
5) Once they have grown a bit, test them for desired characteristics (cytotoxicity, FACS...) and then select them carefully. Once selected, plate only 1 clone/plate.
6) Freeze 2-3 vials/clone as early as possible. Once confluent (medium can get slightly yellowish), harvest plate almost entirely and freeze down in cold freezing medium. Transfer the remaining cells to a fresh plate with fresh medium and feeders.
7) Passage the cells once per week-10 days. When plating, seed at less than 2 x 10^6/plate and allow to grow up to 10 x 10^6.

* Feeder cells can be allogeneic. Freeze aliquots of PBMCs, thaw and then irradiate. For best results, use fresh feeders. Use 2 x 10^4 – 5 x 10^4 PBMCs/well and 1 x 10^4 LCLs. LCLs are very important for establishing clones; less yield is achieved with PBLs alone. Irradiate PBMCs at 5-10 Gey, LCLs at 30 Gey. If only using autologous PBMCs (no LCLs), then use 3-5x10^4 per well.

First Version

Dr. DeKaban wrote comm

Generation of EBV-transformed B cell lines

5ml of complete culture medium containing 1µg/ml Cyclosporin A. Separate clear cells from a minimum of 5 ml of peripheral blood Centrifuge 5×10^6 PBMC nutes at 400 x g. Remove supernatant. Resuspend cells in 1.8 ml of complete ~~nedium containing~~ 1µg Cyclosporin A. Add 100-200µl of the concentrated B95- ~~upernatant~~ to cell mixture. Mix gently. Place 200µl of the cell mixture in each of of a 96-well flat-bottomed plate. Place 100µl of cell mixture into another 10 the same 96 well flat bottom plate. Add an additional 100µl of CSA containing to the 10 wells containing 100µl of cell suspension. The final volume per well is ill the outside wells with sterile water. Place plate(s) in 37°C, 5% CO_2 incubator. ite weekly: Remove plate(s) from incubator and aspirate 0.1 ml of media from ll of the 96-well flat-bottom plate. Add 0.1ml of fresh media to each well and) incubator. After two weeks of incubation, if transformed cell clumps are ıg, combine cells from three wells of the 96-well plate into one well of a 24-well ɔmed plate. Repeat two more times to obtain a total of 3 wells in a 24-well plate. :maining cells in 96-well plate as reserves. Incubate plate at 37°C for one week in ıcubator for further cell expansion. Once cells are clearly proliferating, remove m 24-well plate and place them into a 25 cm^2 vented flask with 5ml complete lus 100µM of Acyclovir. Acyclovir is added to ensure that there will be no s virus present in the culture. Feed cells 1-2 times per week with complete dding 100µM Acyclovir each time. After approximately two weeks, freeze 5 h at least 2×10^6 LCLs. LCLs may be maintained in culture for a maximum of 9 ɔost initiation.

Not listed on protocol. How generated & from where?

Since there is a protocol for transforming B cells with EBV does this mean this is being done in Robarts? If yes source of EBV? who went to be done + by whom? Has Gabriella Sierra gone through and Biosafety training

✓ → levels biosafety cell lines

✓ → certificate from UofT (biosafety)

✓ → course list

✓ → revised LCL protocol

γδ T cell isolation and expansion protocol
Dec 2009

Materials

MACS buffer
AIMV medium + 5% heat inactivated hu serum
ConA
IL-2
IL-4
MACS antibody and beads
Ice
MACS column, magnet and holder
Eppis for counting
24 well plates (Falcon)

1. Isolate PBMCs from donors. Expected yield ~1.0 x 10^6 cells/ml
   COUNT:
2. Spin 5 min at 1800 rpm. Resuspend cells at 40µl/10^7 cells in cold MACS buffer (degassed) in a 50 ml conical.
   µl buffer used:
3. Remove 6µl for FACS analysis.
4. Add γδ TCR hapten antibody at 10µl/10^7 cells. Incubate in the fridge for 10 min.
   µl Ab used:
   incubation time:
5. Wash with 10 ml cold MACS buffer. Spin 5 min 1500 rpm 4 deg. Discard supernatant.
6. Resuspend cells at 30µl/10^7 cells in cold MACS buffer.
   µl buffer used:
7. Add anti-hapten-FITC antibody/beads at 20µl/10^7 cells. Incubate in the fridge for 15 min. µl Ab used:
   incubation time:
8. Wash with 10 ml cold MACS buffer. Spin 5 min 1500 rpm 4 deg.
9. Prime MACS column by inserting it into holder and carefully adding 500µl.
10. Discard supernatant and resuspend cell pellet in 500µl cold MACS buffer and then carefully add to column, by placing tip against the side of the column and allowing the cell suspension to run down the side.
11. Wash column with 3 x 500µl MACS buffer.
12. Add 3 ml medium to column. Remove column from magnetic holder and place into a fresh 15 ml conical. Insert plunger and press down to elute cells from the column.
13. Count cells, resuspend at 2.5 x 10^5 cells/ml and seed into 24-well plates (2ml/well). Use 1-2 ml for FACS analysis (wash in FACS buffer 1 x before antibody incubation).
14. Remove 75µl (15µl/sample) from MACS negative fraction for FACS analysis.

FACS Staining:

	preMACS	MACS+	MACS-
unstained	1	7	9
CD3 FITC 1:100	2	-	10
γδ TCR PE 1:10	3	-	11
αβ TCR PE 1:20	4	8	12
Vδ2 PE 1:100	5	-	
Vδ1 FITC 1:50	6		

Antibodies: 20-50 μl dilution/sample

Important notes:

50 ml conicals for isolation and antibody incubations
25-27 min primary antibody and 15 min secondary antibody incubations for MACS sorting
Con A 7-8 days (8 days better!)
remove all medium and replace with fresh at each feeding
spin down at 800rpm (sugg by Simone) ROOM TEMP
96 or 24 well-plates are better than 6-wells or 12-wells
12 and 6-wells are fine for passages beyond ConA and the first passage after ConA removal
expansion stops in T75 flasks

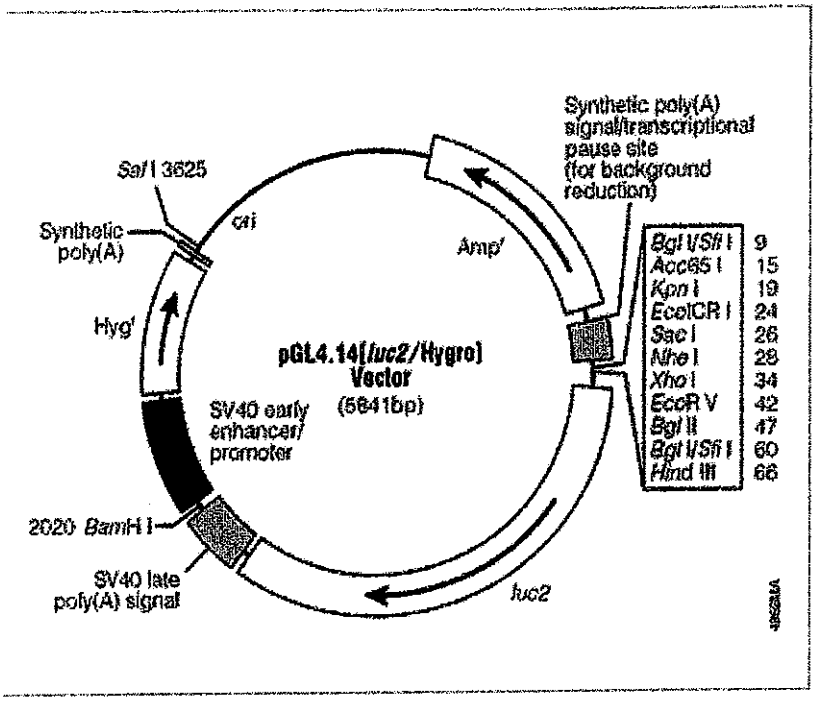
best seeding density 2.5-5 x 10E5 cells/ml
don't change med until med is apricot or yellowish
spin at RT

AIM-V medium (can probably use RPMI)
5% hu AB serum
10 ng/ml IL-2
10 ng/ml IL-4

first 6-8 days:
1μg/ml Concanavalin A

Keep the clones up to 4 weeks in culture after they begin to expand. You cannot say by vision if cells are clonal or polyclonal. As a matter of fact, gamma delta clones typically grow in a "flower-type" shape since the cells show active motility,particularly the acivated clones with high cytotoxic potential...They may however also grow as round colonies if they are longer in culture or if they tend "to be overgrown".

Thawing: Thaw 1 vial in fresh Iscoves Medium with 10% HS no cytokines. Centrifuge cells, then resuspend in complete medium with IL-2, irradiated feeders and PHA. Plate 60 wells on one plate. Paul never counted them after thawing. If it worked you should see growth with naked eyed within 1 to three days... If you have too many clones to thaw you can also plate on ½ plate (30 wells) and expand later once they grow. To improve conditions it helps taking fresh feeder cells and not frozen ones...



Promega Corporation ~ 2800 Woods Hollow Road ~ Madison, WI USA
608-274-4330

Mammalian cell transfection*

*I've used this technique previously for MDA-MB-231BR eGFP cells at Stanford University in Stanford, CA, USA.

** The media used for the below cell line is DMEM with 10% FBS.

*** This will be repeated using the pDsRed Monomer-Hyg-N1 vector. The procedure for other vectors only differs in the selection agent used, please see step 7.

**MDA-MB-231BR eGFP cells

1. Seed 1.25 x 10^5 cells/well in a 6 well plate. For each vector used seed triplicate wells.
2. Allow cells to adhere overnight at 37C, 5% CO_2
3. The following day exchange media with 500 uL of fresh DMEM
4. In separate sterile Eppendorf tubes combine 500ng of plasmid, 100uL of OptiMEM media and 2 uL of Lipofectamine 2000. Make one tube for each well of cells to be transfected. Incubate at room temperature for 30 min.
5. Add full contents of each tube to a separate cell-seeded well. Let incubate overnight at 37C, 5% CO_2
6. After 24h split cells 1:10. Let incubate overnight at 37C, 5% CO_2
7. Add mammalian selection agent to each well. ***For pDsRed Monomer-Hyg-N1 use 125 ug/mL Hygromycin, for pcDNA3.1 or pEYFP use 500ug/mL Geneticin/Neomyocin respectively.
8. Grow cells under selection for 2-3 weeks, changing media every 3-4 days.
9. Once distinct drug-resistant colonies form, pick individual colonies into individual 24 well plate wells. Continue to grow under selection under a large clonal population is formed.
10. 4. To each well of a 24 well plate, add 20 000 cells, 8ug/mL of protamine sulfate (dissolved in PBS), the lentivirus suspension, and top off to a final volume of 1mL.
11. Keep track of cell growth; if necessary, move to larger tissue culture ware. Cells can be stored longterm in liquid nitrogen.

pcDNA3.1(+)
pcDNA3.1(-)

Catalog nos. V790-20 and V795-20, respectively

Version I
081401
28-0104

Invitrogen™
life technologies

www.invitrogen.com
tech_service@invitrogen.com

Table of Contents

Important Information

Contents

pcDNA3.1 is supplied as follows:

Catalog no.	Contents
V790-20	20 µg pcDNA3.1(+), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0
V795-20	20 µg pcDNA3.1(-), lyophilized in TE, pH 8.0 20 µg pcDNA3.1/CAT, lyophilized in TE, pH 8.0

Shipping/Storage

Lyophilized plasmids are shipped at room temperature and should be stored at -20°C.

Product Qualification

Each of the pcDNA3.1 vectors is qualified by restriction enzyme digestion with specific restriction enzymes as listed below. Restriction digests must demonstrate the correct banding pattern when electrophoresed on an agarose gel. The table below lists the restriction enzymes and the expected fragments.

Vector	Restriction Enzyme	Expected Fragments (bp)
pcDNA3.1(+)	*Nhe* I	5428
	Pst I	1356, 4072
	Sac I	109, 5319
pcDNA3.1(-)	*Nhe* I	5427
	Pst I	1363, 4064
	Sac I	169, 5258
pcDNA3.1/CAT	*Nhe* I	6217
	Pst I	2145, 4072
	Sac I	109, 6008

Methods

Overview

Introduction

pcDNA3.1(+) and pcDNA3.1(-) are 5.4 kb vectors derived from pcDNA3 and designed for high-level stable and transient expression in mammalian hosts. High-level stable and non-replicative transient expression can be carried out in most mammalian cells. The vectors contain the following elements:

- Human cytomegalovirus immediate-early (CMV) promoter for high-level expression in a wide range of mammalian cells
- Multiple cloning sites in the forward (+) and reverse (-) orientations to facilitate cloning
- Neomycin resistance gene for selection of stable cell lines
- Episomal replication in cells lines that are latently infected with SV40 or that express the SV40 large T antigen (e.g. COS-1, COS-7)

The control plasmid, pcDNA3.1/CAT, is included for use as a positive control for transfection and expression in the cell line of choice.

Experimental Outline

Use the following outline to clone and express your gene of interest in pcDNA3.1.

1. Consult the multiple cloning sites described on pages 3-4 to design a strategy to clone your gene into pcDNA3.1.
2. Ligate your insert into the appropriate vector and transform into *E. coli*. Select transformants on LB plates containing 50 to 100 µg/ml ampicillin.
3. Analyze your transformants for the presence of insert by restriction digestion.
4. Select a transformant with the correct restriction pattern and use sequencing to confirm that your gene is cloned in the proper orientation.
5. Transfect your construct into the mammalian cell line of interest using your own method of choice. Generate a stable cell line, if desired.
6. Test for expression of your recombinant gene by western blot analysis or functional assay.

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(+)

Below is the multiple cloning site for pcDNA3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(+) is available for downloading from our web site** (www.invitrogen.com) **or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(+), please refer to the **Appendix**, pages 10-11.

enhancer region (3′ end)

```
689  CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG
                          CAAT                                    TATA
749  TAACAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
          3' end of hCMV          putative transcriptional start
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
     T7 promoter/primer binding site        Nhe I      Pme I  Afl II Hind III  Asp718 I  Kpn I
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
     BamH I                    BstX I*  EcoR I      EcoR V      BstX I*  Not I     Xho I
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
        Xba I        Apa I  Pme I                pcDNA3.1/BGH reverse priming site
989  AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
                BGH poly (A) site
1109 TCCTTTCCTA ATAAAATGAG GAAATTGCAT
```

*Please note that there are two *Bst*X I sites in the polylinker.

continued on next page

Cloning into pcDNA3.1, continued

E. coli Transformation

Transform your ligation mixtures into a competent *rec*A, *end*A *E. coli* strain (e.g. TOP10F′, DH5α™-T1^R, TOP10) and select transformants on LB plates containing 50 to 100 µg/ml ampicillin. Select 10-20 clones and analyze for the presence and orientation of your insert.

We recommend that you sequence your construct with the T7 Promoter and BGH Reverse primers (Catalog nos. N560-02 and N575-02, respectively) to confirm that your gene is in the correct orientation for expression and contains an ATG and a stop codon. Please refer to the diagrams on pages 3-4 for the sequences and location of the priming sites. The primers are available separately from Invitrogen in 2 µg aliquots.

Preparing a Glycerol Stock

Once you have identified the correct clone, purify the colony and make a glycerol stock for long-term storage. You should keep a DNA stock of your plasmid at -20°C.

- Streak the original colony out on an LB plate containing 50 µg/ml ampicillin. Incubate the plate at 37°C overnight.
- Isolate a single colony and inoculate into 1-2 ml of LB containing 50 µg/ml ampicillin.
- Grow the culture to mid-log phase (OD_{600} = 0.5-0.7).
- Mix 0.85 ml of culture with 0.15 ml of sterile glycerol and transfer to a cryovial.
- Store at -80°C.

Creation of Stable Cell Lines

Introduction

The pcDNA3.1(+) and pcDNA3.1(-) vectors contain the neomycin resistance gene for selection of stable cell lines using neomycin (Geneticin®). We recommend that you test the sensitivity of your mammalian host cell to Geneticin® as natural resistance varies among cell lines. General information and guidelines are provided in this section for your convenience.

Geneticin® Selective Antibiotic

Geneticin® Selective Antibiotic blocks protein synthesis in mammalian cells by interfering with ribosomal function. It is an aminoglycoside, similar in structure to neomycin, gentamycin, and kanamycin. Expression of the bacterial aminoglycoside phosphotransferase gene (APH), derived from Tn*5*, in mammalian cells results in detoxification of Geneticin® (Southern and Berg, 1982).

Geneticin® Selection Guidelines

Geneticin® Selective Antibiotic is available from Invitrogen (Catalog no. 10486-025). Use as follows:

- Prepare Geneticin® in a buffered solution (e.g. 100 mM HEPES, pH 7.3).
- Use 100 to 800 µg/ml of Geneticin® in complete medium.
- Calculate concentration based on the amount of active drug (check the lot label).
- Test varying concentrations of Geneticin® on your cell line to determine the concentration that kills your cells (see below). Cells differ in their susceptibility to Geneticin®.

Cells will divide once or twice in the presence of lethal doses of Geneticin®, so the effects of the drug take several days to become apparent. Complete selection can take up to 3 weeks of growth in selective media.

Determination of Antibiotic Sensitivity

To successfully generate a stable cell line expressing your gene of interest from pcDNA3.1, you need to determine the minimum concentration of Geneticin® required to kill your untransfected host cell line. We recommend that you test a range of concentrations to ensure that you determine the minimum concentration necessary for your host cell line.

1. Plate or split a confluent plate so the cells will be approximately 25% confluent. Prepare a set of 7 plates. Allow cells to adhere overnight.
2. The next day, substitute culture medium with medium containing varying concentrations of Geneticin® (0, 50, 100, 200, 400, 600, 800 µg/ml Geneticin®).
3. Replenish the selective media every 3-4 days, and observe the percentage of surviving cells.
4. Count the number of viable cells at regular intervals to determine the appropriate concentration of Geneticin® that prevents growth within 2-3 weeks after addition of Geneticin®.

continued on next page

Creation of Stable Cell Lines, continued

Selection of Stable Integrants

Once you have determined the appropriate Geneticin® concentration to use for selection in your host cell line, you can generate a stable cell line expressing your gene of interest.

1. Transfect your mammalian host cell line with your pcDNA3.1 construct using the desired protocol. Remember to include a plate of untransfected cells as a negative control and the pcDNA3.1/CAT plasmid as a positive control.
2. 24 hours after transfection, wash the cells and add fresh medium to the cells.
3. 48 hours after transfection, split the cells into fresh medium containing Geneticin® at the pre-determined concentration required for your cell line. Split the cells such that they are no more than 25% confluent.
4. Feed the cells with selective medium every 3-4 days until Geneticin®-resistant foci can be identified.
5. Pick and expand colonies in 96- or 48-well plates.

pcDNA3.1 Vectors, continued

Features of pcDNA3.1(+) and pcDNA3.1(-)

pcDNA3.1(+) (5428 bp) and pcDNA3.1(-) (5427 bp) contain the following elements. All features have been functionally tested.

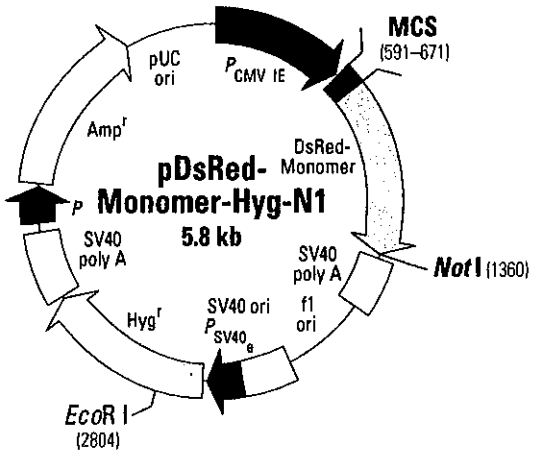
Feature	Benefit
Human cytomegalovirus (CMV) immediate-early promoter/enhancer	Permits efficient, high-level expression of your recombinant protein (Andersson *et al.*, 1989; Boshart *et al.*, 1985; Nelson *et al.*, 1987)
T7 promoter/priming site	Allows for *in vitro* transcription in the sense orientation and sequencing through the insert
Multiple cloning site in forward or reverse orientation	Allows insertion of your gene and facilitates cloning
Bovine growth hormone (BGH) polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA (Goodwin and Rottman, 1992)
f1 origin	Allows rescue of single-stranded DNA
SV40 early promoter and origin	Allows efficient, high-level expression of the neomycin resistance gene and episomal replication in cells expressing SV40 large T antigen
Neomycin resistance gene	Selection of stable transfectants in mammalian cells (Southern and Berg, 1982)
SV40 early polyadenylation signal	Efficient transcription termination and polyadenylation of mRNA
pUC origin	High-copy number replication and growth in *E. coli*
Ampicillin resistance gene (β-lactamase)	Selection of vector in *E. coli*

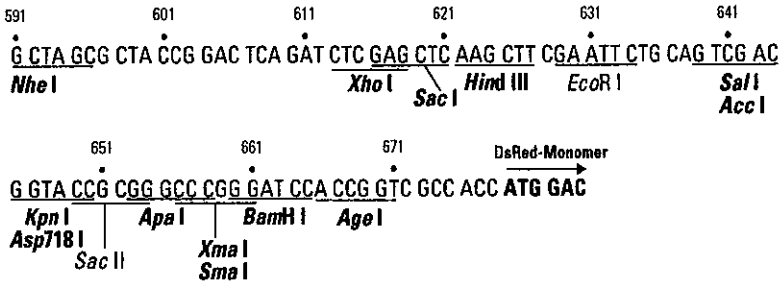
Vector Information

pDsRed-Monomer-Hyg-N1 Vector Information

PT3843-5

Cat. No. 632494





Restriction Map and Multiple Cloning Site (MCS) of pDsRed-Monomer-Hyg-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed-Monomer stop codon. NOTE: The *Xba* I and *Bcl* I sites are methylated in the DNA provided by Clontech Laboratories, Inc. If you wish to digest the vector with these enzymes, you will need to transform the vector into a dam– host and make fresh DNA.

Description

pDsRed-Monomer-Hyg-N1 is a mammalian expression vector that encodes DsRed-Monomer (DsRed.M1), a monomeric mutant derived from the tetrameric *Discosoma sp.* red fluorescent protein DsRed (1). DsRed-Monomer contains forty-five amino acid substitutions (listed on page 2). When DsRed-Monomer is expressed in mammalian cell cultures, red fluorescent cells can be detected by either fluorescence microscopy or flow cytometry 12–16 hr after transfection (DsRed-Monomer excitation and emission maxima = 557 nm and 592 nm, respectively). The DsRed-Monomer coding sequence is human codon-optimized for high expression in mammalian cells (2).

DsRed-Monomer is well suited for use as a fusion tag. The multiple cloning site (MCS) in pDsRed-Monomer-Hyg-N1 is positioned between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed-Monomer coding sequence. Genes cloned into the MCS are expressed as fusions to the N-terminus of DsRed-Monomer if they are in the same reading frame as DsRed-Monomer and there are no intervening stop codons. A Kozak consensus sequence is located immediately upstream of the DsRed-Monomer gene to enhance translational efficiency in eukaryotic systems (3). SV40 polyadenylation signals downstream of the DsRed-Monomer gene direct proper processing of the 3' end of the DsRed-Monomer mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen, a pUC origin of replication for propagation in *E. coli*, and an f1 origin for single-stranded DNA production. A hygromycin resistance cassette (Hyg^r)—consisting of the SV40 early promoter, the hygromycin resistance gene, and SV40 polyadenylation signals—allows stably transfected eukaryotic cells to be selected using hygromycin. A bacterial promoter-resistance gene cassette confers ampicillin resistance in *E. coli*.

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E-mail: tech@clontech.com
www.clontech.com

GTG→TGC (Val-175 to Cys) mutation: 1201–1203
GAG→GAC (Glu-176 to Asp) mutation: 1204–1206
TCC→ACC (Ser-179 to Thr) mutation: 1213–1215
ATC→GTG (Ile-180 to Val) mutation: 1216–1218
ATG→AAG (Met-182 to Lys) mutation: 1222–1224
TAC→AAC (Tyr-192 to Asn) mutation: 1252–1254
TAC→CAC (Tyr-193 to His) mutation: 1255–1257
TCC→AAC (Ser-203 to Asn) mutation: 1285–1287
ATC→GTG (Ile-210 to Val) mutation: 1306–1308
CGC→CAC (Arg-216 to His) mutation: 1324–1326
ACC→GCC (Thr-217 to Ala) mutation: 1327–1329
GGC→GCC (Gly-219 to Ala) mutation: 1333–1335
CAC→TCC (His-222 to Ser) mutation: 1342–1344
CTG→GGC (Leu-223 to Gly) mutation: 1345–1347
TTC→TCC (Phe-224 to Ser) mutation: 1348–1350
CTG→CAG (Leu-225 to Gln) mutation: 1351–1353

- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1510–1515 & 1539–1544; mRNA 3' ends: 1548 & 1560
- f1 single-strand DNA origin: 1607–2062 (Packages the noncoding strand of DsRed-Monomer)
- SV40 origin of replication: 2403–2538
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2236–2307 & 2308–2379
  21-bp repeats: 2383–2403, 2404–2424 & 2426–2446
  Early promoter element: 2459–2465
  Major transcription start points: 2455, 2493, 2499 & 2504
- Hygromycin resistance gene:
  Start codon (ATG): 2560–2562; stop codon: 3583–3585
- SV40 early mRNA polyadenylation signal: 3732–3737 & 3761–3766; mRNA 3' ends: 3770 & 3782
- Bacterial promoter for expression of Amp^r gene:
  –35 region: 3932–3937; –10 region: 3955–3960
- Ampicillin resistance gene:
  Start codon (ATG): 4002–4004; stop codon: 4860–4862
- pUC plasmid replication origin: 5025–5668

Sequencing primer location

- DsRed1-N Sequencing Primer (Cat. No. 632387; 5'-GTACTGGAACTGGGGGGACAG-3'): 879–859

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid, such as the JM109 or XL1-Blue strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: high

Excitation and emission maxima of DsRed-Monomer

- Excitation maximum = 557 nm
- Emission maximum = 592 nm

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.

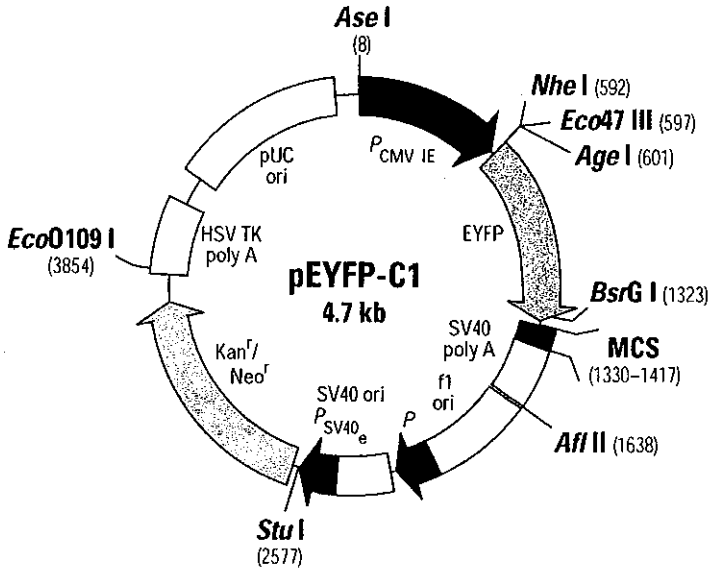
Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by Clontech Laboratories, Inc. This vector has not been completely sequenced.

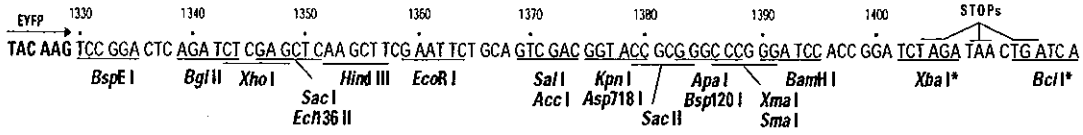
Vector Information

pEYFP-C1 Vector Information

PT3176-5

Catalog #6005-1





Restriction map and multiple cloning site (MCS) of pEYFP-C1. All restriction sites are shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-C1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513-nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-C1 is between the EYFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening in-frame stop codons. EYFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neo^r) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neo^r expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The recombinant EYFP vector can be

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Spin Infection

** This was done using the pCCL-EGFP-minCMV-hPGK-Luc lentiviral vector

*** Media used: PC3M cells used RPMI 1640 with 10% FBS, L-glutamine, Pen-Strep, 1x HEPES, and non-essential amino acids.

PC3M cells

1. Make sure the cells are growing nicely in log phase prior to transduction.
2. 2 days prior to transduction, seed a T25 flask approximately 1:4 from an 80% confluent flask.
3. On the day of transduction, lift the cells and count.
4. To each well of a 24 well plate, add 20 000 cells, 8ug/mL of protamine sulfate (dissolved in PBS), the lentivirus suspension, and top off to a final volume of 1mL.

1. The amount of lentivirus suspension to use depends on the multiplicity of infection you intend to go with. I used an MOI of 5 and got adequate levels of transductants.

1. The MOI refers to the number of transducing units per every cell. So an MOI of 5 with 20 000 cells meant 100 000TU

5. Spin down in the Beckman GS-15 centrifuge using the plate rotor

2. 2h30mins, 1000 x g, at room temperature, no brakes

1. I didn't try it with brakes

3. You'll need prewarmed media after this, so get some ready in the last hour of spinning.

6. Remove media and replace with a fresh 1mL of prewarmed media (per well)
7. Incubate at 37C, 5% CO2 for at least 48 hours to allow for integration and expression
8. Keep track of cell growth; if necessary, move to larger tissue culture ware

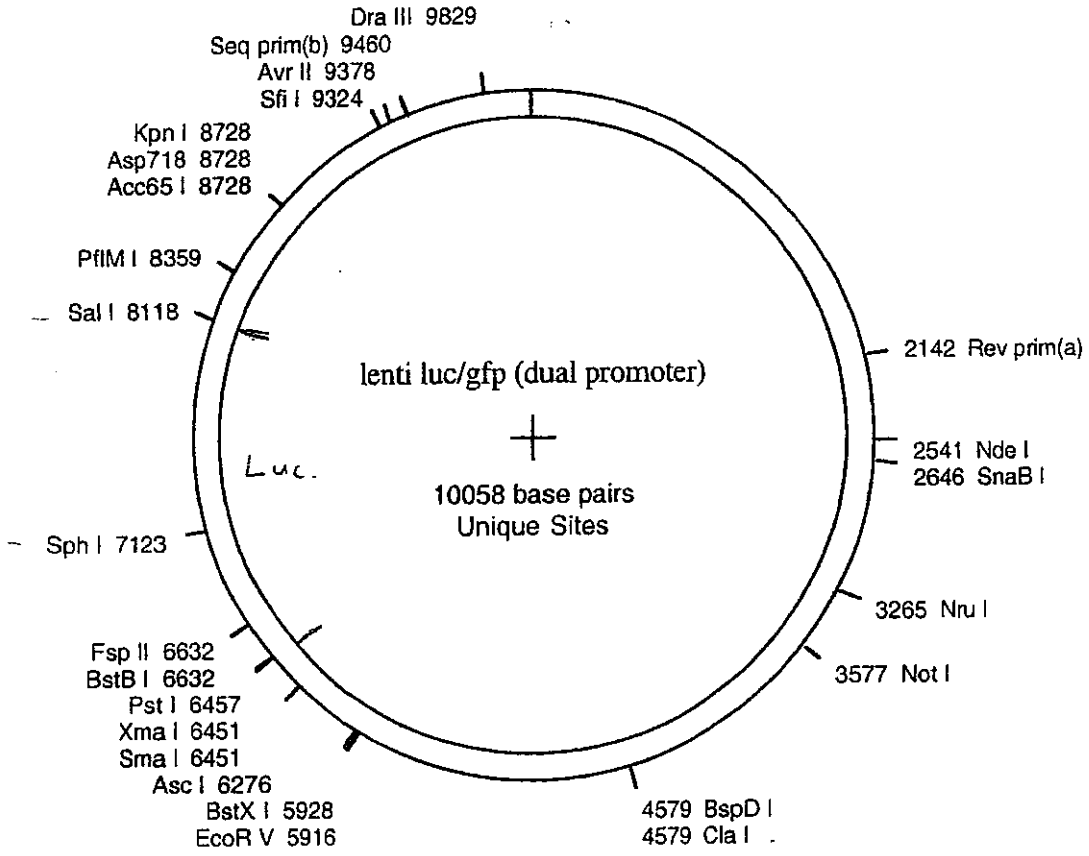
1. I didn't want to throw out any cells, so I lifted them with trypsin, and replated everything into a 6 well plate. Once that was confluent, it was moved to a T25, and lastly to a T75 as confluency dictated.

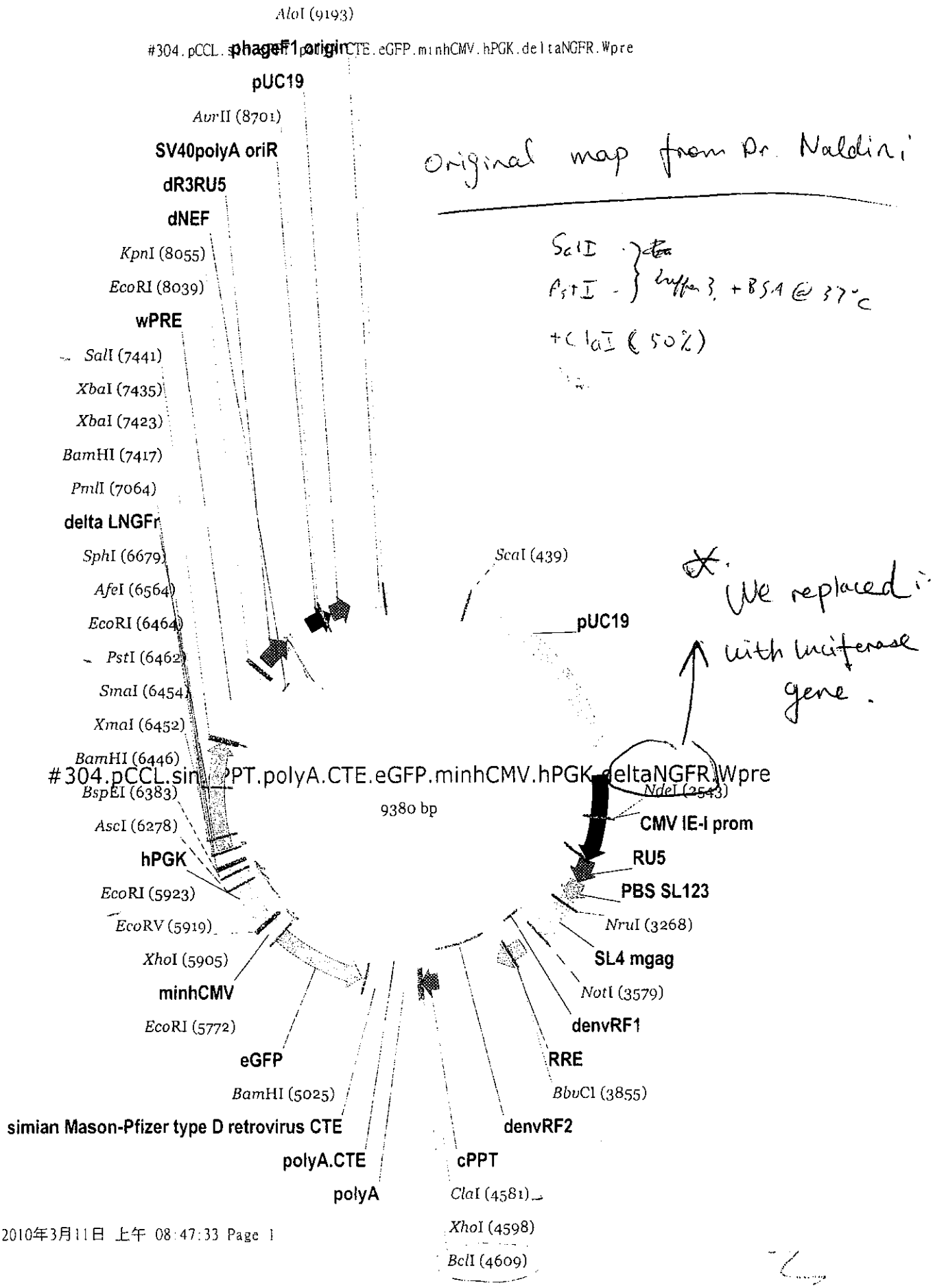
DNA Strider™ 1.2.1 ### Friday, March 31, 2006 10:07:19 AM

lenti luc/gfp (dual promoter) -> Graphic Map

DNA sequence 10058 b.p. caggtggcactt ... tttacaatttcc circular

#304.pCCL.sin.cPPT.polyA.CTE.eGFP.minhCMV.hPGK.luc.Wpre
PGKpromoter= 5928-6443 luc= 6464-8117 (PstI/SalI)
minhCMVpromoter=5786-5894 (reverse) eGFP=5051-5767 (reverse)





Original map from Dr. Naldini

SalI } ctm
PstI } buffer 3 + BSA @ 37°C
+ ClaI (50%)

* We replaced it with luciferase gene.

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