Biohazards Committee

Permit Holder: H.G. Hedrick

Approved Personnel
(Please stroke out any personnel to be removed)

Faran Vafaie

Oula Akawi

Theo Small

Caroline Oneil

Zengxuan Nong

Matt Frontini

Nica Borradaile

Alanna Watson

Additional Personnel
(Please list additional personnel here)

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E.coli, alpha competent cells	
Approved Cells	Human (established) HEK 293, Human skin fibroblast, Transformed human skin fibroblast, human aortic endothelial cells, enca cells, CH3/10T 1/2, 3T3-L1, 3T3-Swiss Albino, cos-7, HeLa, HAEC, HT-1080	
Approved Use of Human Source Material	Primary smooth muscle cells	
Approved GMO	Adeno E1A (HEK 293), Admax (Adeno), vectors- pD3111, pDC411, pDC511, pLNCX chick src Y527F plasmid, pcDNA3 Flag FKHR AAA mutant plasmid (FOX O1A), pEGfP-N3, pIRES2-EGFP, pLNCX2,	pQCXIP-Nampt-IRES-PURO pQCXIP-IRES-PURO pBluescriptSKII

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 25, 2007

Signature of Permit Holder: _____

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

[redacted]

Approved use of Animals	Rats, mice	
Approved Toxin(s)		

** PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
*** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Jan 25, 2007

Signature of Permit Holder: J. R. Kile

BioSafety Officer(s): _____

Chair,Biohazards Subcommittee: _____

Modification Form For Permit BIO-RRI-0028
Permit Holder: J.G Pickering

pQCXIP-Nampt-IRES-PURO and pQCXIP-IRES-PURO constructs

These plasmids are both used within the Phoenix system by the Nolan lab at Stanford University (more information about this system can be found at http://www.stanford.edu/group/nolan/retroviral_systems/phx.html)
pQCXIP-IRES-PURO is used as a control vector in the phoenix system to transducer mammalian cells with puromycin resistance. pQCXIP-Nampt-IRES-PURO is used in the phoenix system to transduce mammalian cells with the protein Nampt (nicotinamide phosphoribosyltransferase) and puromycin resistance.
For more details regarding the protocols in which these plasmids are used please refer to the following website:
http://www.stanford.edu/group/nolan/protocols/pro_helper_dep.html

pSPTg.TFpAXK, pSPTg.T2FXK, pHHSDKXK, pT2HLacZ, pAII.7 , pg SD-2.11

(All are from the parental vector pBluescriptSKII)

The "vascular endothelial promoter/enhancer package" is a set of DNA plasmids deisgned by the Sato lab to specifically express a gene of interest in the vascular endothelium of mice (Schlaeger TM, et al., PNAS, 1997). We will use these plasmids to generate transgenic mice expressing our gene of interest (nicotinamide phosphoribosyltransferase) only in endothelial cells of blood vessel walls.
For plasmid maps and details please refer to the following website:
http://www.cornellcelldevbiology.com/sato/reagents/utsw/dna/dna_reagents_page.htm

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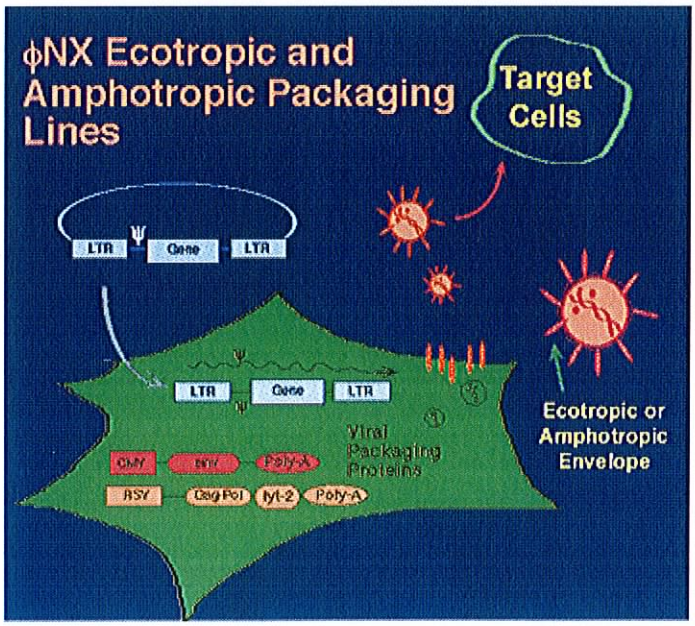
Phoenix System > Episomes > Tetracycline Regulated

Phoenix helper-free retrovirus producer lines:

Phoenix is a second-generation retrovirus producer lines for the generation of helper free ecotropic and amphotropic retroviruses. The lines are based on the 293T cell line (a human embryonic kidney line transformed with adenovirus E1a and carrying a temperature sensitive T antigen co-selected with neomycin).

The unique feature of this cell line is that it is highly transfectable with either calcium phosphate mediated transfection or lipid-based transfection protocols-- up to 50% or higher of cells can be transiently transfected.

The lines were created by placing into 293T cells constructs capable of producing gag-pol, and envelope protein for ecotropic and amphotropic viruses. The lines offered advantages over previous stable systems in that virus can be produced in just a few days.



The lines have several other improvements over other approaches and this has led to their broad application worldwide:

- We added the facility to monitor gag-pol production on a cell-by cell basis by introducing an IRES-CD8

Related links:

Download Material Transfer Agreements for Phoenix lines.

Use of EBV based Episomes with Retroviruses

Tetracycline regulated retroviruses

surface marker downstream of the reading frame of the gag-pol construct. Thus, CD8 expression is a direct reflection of intracellular gag-pol and the stability of the producer cell population's ability to produce gag-pol can be readily monitored by flow cytometry.

- For both the gag-pol and envelope constructs non-moloney promoters were used to minimize recombination potential.
- Different promoters for gag-pol and envelope were used to minimize their inter-recombination potential.
- Two cell lines were created, Phoenix-Eco and Phoenix-Ampho. Gag-pol was introduced with hygromycin as the co-selectable marker and the envelope proteins were introduced with diptheria resistance as the co-selectable marker.
- Finally, a line is available that expresses only gag-pol; this line is termed Phoenix-gp. This line is available for further pseudotyping of retroviral virions with other envelope proteins such as gibbon ape leukemia virus envelope or Vesicular stomatitus VSV-G protein.
- Both Phoenix-Eco and Phoenix-Ampho have been extensively tested for helper virus production and established as being helper-virus free.
- Both lines are capable of carrying episomes for long-term stable production of retrovirus (LZRS).

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Related links:

Phoenix - Helper dependent protocol

Preparing Retrovirus using Phoenix Lines. This is one of two transfection protocols you can find in this web page set.

IMPORTANT NOTE

The viral supernatants produced by these methods might,depending upon your retroviral insert, contain potentially hazardous recombinant virus. The user of these systems must exercise due caution in the production, use and storage of recombinant retroviral virions, especially those with amphotropic and polytropic host ranges. This consideration should be applied to all genes expressed as amphotropic and polytropic pseudotyped retroviral vectors. Appropriate NIH and other regional guidelines should be followed in the use of these recombinant retrovirus production systems. The user is strongly advised NOT to create retroviruses capable of expressing known oncogenes in amphotropic or polytropic host range viruses.

Phoenix Retroviral Producer Line Protocol

{Day 0 | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | X-Gal Staining}

Day 0: Preparation of Phoenix Retrovirus Producer cells for Transfection.

- 18-24 hours prior to transfection, plate Phoenix cells at 1.5-2 million cells per 6 cm plate in producer cell growth media.
- After adding cells, set plates in incubator and gently shake forward and backward, then side to side, 3-4 times. This distributes cells evenly about the plate. Do not disturb cells for several hours while they attach to plate.
- Cells are allowed to attach for 10 hours to 24 hours on 10 or 15 cm plates.
- At 2/3 confluence, a 10 cm plate should provide 5 million cells, a 15 cm plate will contain about 7.5-15 million cells. It is at this subconfluent stage that cells are most transfectable and will survive the rigours of

transfection best, giving the highest titer virus possible.

- **Media:** DMEM, 10% FCS, 1% Penicillin-Streptomycin, 1% Glutamine.
- **Passaging Phoenix cells:**
  - Never let cells reach confluence. This will reduce transfection efficiency in the short term. For maximally healthy cells a split of 1:4 or 1:5, of a 70-80% confluent 10 cm plate, into a new plate every 2-3 days should provide optimal cell conditions. Local media and serum conditions will vary.
  - Passage of Phoenix™ cells every few months in Hygromycin (at 300 ug/ml) and Diptheria Toxin (1 ug/ml) for one week is recommended. Cells can be analyzed and sorted by FACS for expression of CD8 (a proxy measure of gag-pol in this cell line) and for surface expression of envelope protein with 83A25 antibody (see Chesboro et al (ref)).
  - Two million cells on a 60 mm plate is a good starting point for seeding Phoenix cells prior to transfection; it is important to titer slightly up and slightly down to maximize transfection efficiencies. Efficiencies of 50-60% as determined by X-gal staining of the Phoenix cells should be achieved. We have found that the highest transfection efficiencies are obtained with Phoenix cells that are 70-80% confluent at the time of transfection.

Day 1: Transfection

- Prepare the DNA in HBS for application to cells. 1. About 5 minutes prior to transfection, add chloroquine to each plate to 25uM (chloroquine stock is 50 mM; for 3 mL media + 1 mL DNA, add 2u1).
  - Chloroquine acts to inhibit lysosomal DNases by neutralizing vesicle pH.
  - DNA delivered by Ca2PO4 transfection is thought to transit through lysosomes.

- To a 15 mL tube, add (per 6 cm plate, with all reagents at room temperature):
  - 5-10 ug DNA (DNA is added in a drop to side of tube).
  - 438 u1 dd H2O (wash the DNA to bottom of tube with water).
  - 61 ul 2M CaC12 (from Mallinkrodt) -mix thoroughly with finger tapping.
  - 500 ul total volume.

- Scale volume and DNA/reagent amounts if necessary.

- Add 0.5mL 2xHBS quickly then bubble vigourously with automatic pipettor (keep eject button depressed) for 3 - 15 sec (actual length of bubbling time depends on each batch of 2xHBS).
- Add HBS/DNA solution dropwise onto media (gently and quickly) by spreading across cells in media.
- Observe the cells under a microscope; you should observe evenly distributed VERY small black particles.
- Put plate(s) in 37 C incubator; rock plates foreward and backward/back and forth a few times to evenly distribute DNA/CaPO4 particles.
- **HEPES** for Calcium Phospahte Coprecipitation Transfection
  - 1. Make a stock solution of Na2HPO4 dibasic (5.25 g in 500 ml of water)
  - 2. Make 2 x HBS: 8.0 g NaCl 6.5 g HEPES (sodium salt) 10 ml Na2HPO4 stock solution
  - 3. pH to 7.0 using NaOH or HCl. Bring volume up to 500 mls. Check pH again. The pH is very important, it must be exactly 7.0

- **Additional Notes:**
  - 1. HEPES is from Sigma (catalog # H-7006)
  - 2. CaCl2 is from Mallinkrodt (catalog # 4160)
  - 3. Because pH is so important make 3 batches pH 6.95, pH 7.00, pH 7.05. Test each solution and use the one that yields the best precipitate.
  - 4. All reagents should be at room temperature prior to use.

- Please e-mail any questions to Angelica Trejo: angtrejo@stanford.edu

Day 2: 24 hours post-transfection

- Detoxify media and prepare target cells
  - Change media to 3 mL fresh DMEM, 10% FCS
  - Virus is more stable if incubation is carried out at 32 Celsius, although 37 is okay.
  - Do not leave chloroquine on cells more than 24 hours. It is toxic.
  - Prepare for Titering if necessary

- Split NIH 3T3 cells at 200,000 per 6 cm plate in DMEM, 10% CS (1% PentStrep, 1 % Glut).
- If using suspension cells, they should be growing in log phase at time of infection (see below--for Jurkats, ideal density at time of infection is 5E+5 /mL).

Day 3: 48 hours post-transfection - infecting cells with retroviral supernatent

- Pipette supernatant from transfected Phoenix cells into 15 mL tubes and centrifuge at 1500 rpm for 5 minutes to pellet cell debris.
    - Filtering through 0.45 um filter removes cells as well.
    - Supernatant can be frozen at -80 C for later infection, although titer drops by one-half for each freeze-thaw cycle.
- Stain Phoenix cells with X-gal to gauge transfection efficiency
    - This only works if you used a lacZ or other stainable reporter virus).
    - If your virus does not contain such a marker, you can dope the transfection with about 1/10 molar plasmid of a marker that will not interfere with your experiment).
- Remove 1 mL media from each 3T3 plate.
- Add 3 uL polybrene (polybrene is lOOOx at 5 mg/ml) to each 3T3 plate; with gentle and thorough shaking.
- Adherent Cells:
    - Add 1 mL viral supernatant to each 3T3 plate and place at 32/37 Celsius with gentle shaking.
    - Incubate for 8-24 hours, spin cells, and wash away virus supernatent.
- Suspension cells:
    - Pellet 5x10^5 suspension cells, resuspend cell pellet in 1 mL virus + 1 uL lOOOx polybrene.
    - Suspension cells, especially some B cells and T cells are sensitive to polybrene
    - It may be necessary to titrate polybrene to lower levels.

Day 4: Remove Virus Supernatent.

- 24 hours post-infection: change media on 3T3s to fresh DMEM, 10% CS and place at 37 Celsius.
    - For suspension cells, spin out of media and resuspend in 2 mL fresh media.
- Place at 37 Celsius.

Day 5: 24- 48 hours post-infection

- Cells are now ready to assay for biochemical event of interest.
    - The actual reverse transcription and integration take place within 24-36 hours, depending on cell growth kinetics.
    - Expression can start to be observed at 24 hours, usually maxing out at 48 hours.
    - From there on in continued retroviral expression might drop over a period of weeks to months, depending on cell line, site of integration, relative toxicity of insert and a whole bunchof things nobody really understands.
- Your on your own from here. However, if you did add a reporter enzyme, such as lacZ, you can stain 3T3s or supsension cells with X-gal or prepare them for FACS-Gal.

X-gal Staining

- Prepare Solutions:
    - **Fixative**
        - PBS/0.05%
        - Glutaraldehyde (Glutaraldehyde is 25% stock/500x, from Sigma)
    - **Staining solution**
        - per 3 mL ferri/ferrocyanide solution
            - 40-50 uL X-Gal (40mg/mL in DMSO--store at -20oC in the dark).
        - 25X ferricyanide solution:
            - 300mM [K3Fe(CN)6]
            - 130mM MgCl2 in ddH2O
        - 25X ferrocyanide solution:
            - 300mM [K4Fe(CN)6]
            - in ddH2O.
        - Protect the solutions from exposure to light and store at 4oC.
    - **Washing solution**
        - PBS
        - For nonadherent cells add FCS to 1-5%
- Remove media from adherent cells or spin down nonadherent cells in 15mL conical tube.
- Add 2 mL fixative to 6cm plate of adherent cells or resupsend nonadherent cells in 1 mL fixative. Leave for 1 min.
    - For adherent cells, remove and wash 3xPBS (first two washes are quick, third is for 3 min).
    - For nonadherent cells, quench fixative by adding 5-lOmL PBS/1-5% FCS to conical and spin down again.
- Layer 3mL staining solution onto adherent cells or resuspend nonadherent cells in 1 mL staining solution and place in well of 24well plate.
- Optimal staining will occur 24 hours later. If longer,

remove staining solution from cells at 24 hours and re-layer/resupsuspend in ferri/ferrocyanide solution without X-Gal.

- **Milder Fixative:**
  - 2% Paraformaldehyde/0.2% Glutaraldehyde
  - Should be left on cells for 2 min and washed 3xfast with PBS.
  - It is much more difficult to prepare:
    - 1. 4% paraformaldehyde stock: in fume hood, dissolve 8g powder in 150mL of 0.1M sodium phosphate pH 7.3 (66mM NA2HPO4 or 33mM NAH2PO4), stirring and heating at 60oC
    - Add 10N NaOH at rate of 1 drop/min until solution clears. Bring up volume to 200mL with 0.1 M sodium phosphate pH 7.3. Store at 4 C for up to 1 month.
    - Combine 50mL 4% paraformaldehyde solution with 49.2 mL 0.1M sodium phosphate pH 7.3 and 0.8mL 25m% glutaraldebyde. Store at 4 for up to 1 week.

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THE UNIVERSITY OF TEXAS
Southwestern Medical center
At Dallas

Thomas N. Sato, Ph.D.
Professor

Molecular Cardiology Laboratories
Departments of Internal Medicine and
Molecular Biology and Oncology

Dear Colleague:

Enclosed please find the following plasmid DNAs necessary for expressing genes specifically in endothelial cells of transgenic mice:

(#52) pSPTg.T2FpAXK: This vector contains Tie2 promoter, SV40 polyA signal and Tie2 minimum enhancer fragment. MCS are located between the promoter and pA signal where you can insert your cDNA as indicated. DNA fragments can be excised by SalI digest for injection.

(#54) pSPTg.T2FXK: Same as pSPTg.T2FpAXK except that there is no SV40 polyA signal fragment.

(#19) pHHSDKXK: Positive control DNA where LacZ is expressed under the control of theTie2 promoter and minimum enhancer fragment. The insert DNA fragment (approximately .6kb) can be excised by KpnI digest.

(#59) pT2HLacZpA11.7: Positive control DNA where LacZ is expressed under the control of Tie2 promoter and enhancer using the longer enhancer fragment as indicated in the map. Insert DNA fragments can be excised by SalI digest.

(#65) pg50-2.11: TIE2 genomic subclone DNA containing the 10kb enhancer fragment (NaeI-SalI or NotI).

****DNA provided at 1ng/μl in 10μl $TE^{1/10}$ ****

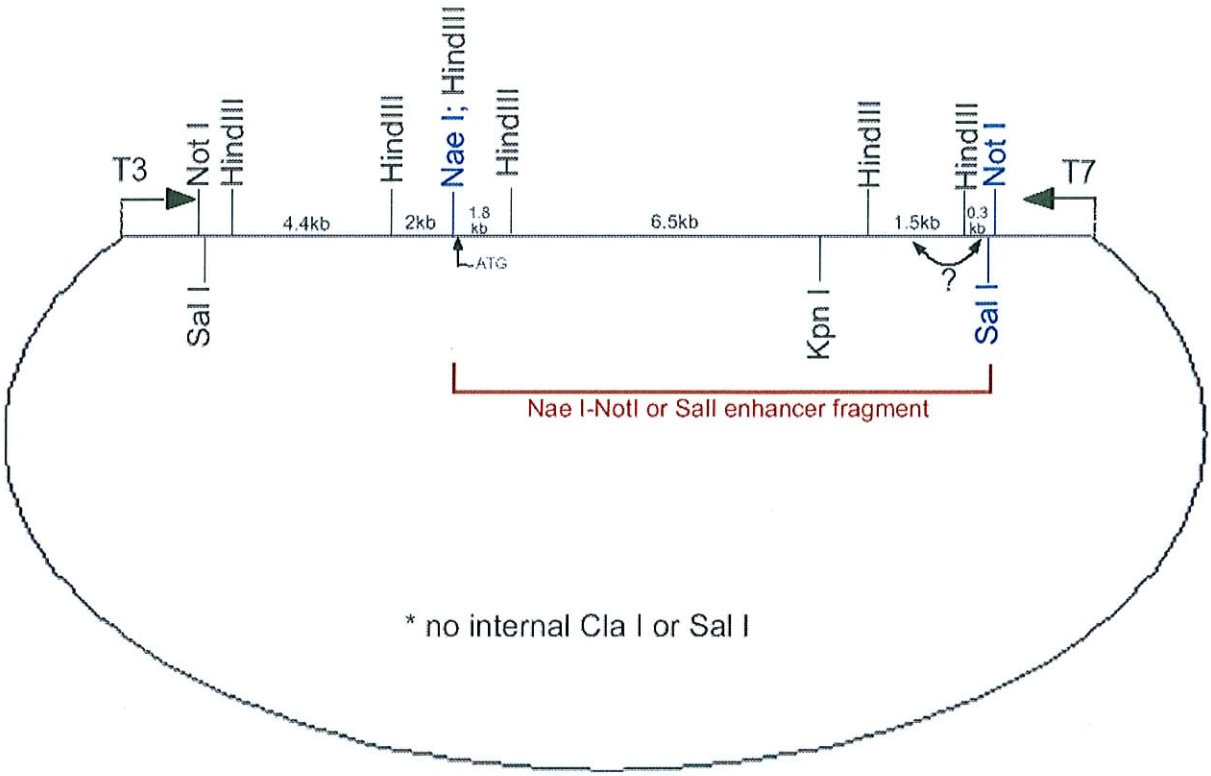
As noted in the PNAS paper, it is essential to use the longer enhancer fragment to obtain the uniform vascular expression, especially in adult. To obtain such a construct, you should digest the "pT2HLacZpA11.7". DNA with XbaI(partial digest) and then, NotI or SalI digest to isolate pA and the enhancer fragment for subcloning or, as an alternative, use the NaeI-SalI (or Not1) fragment from the pg50-2.11 as the enhancer fragment for subcloning.

I would like to ask you to restrict the use of these reagents to the purpose you described in your letter and within your laboratory. If you have any questions, please do not hesitate to contact me. E-mail or Fax is preferred.

Sincerely,

Tom Sato

Southwestern Medical School 5323 Harry Hines Blvd./Dallas, Texas 75390-8573/ (214)648-1402 Fax (214)648-1433/island1005@aol.com



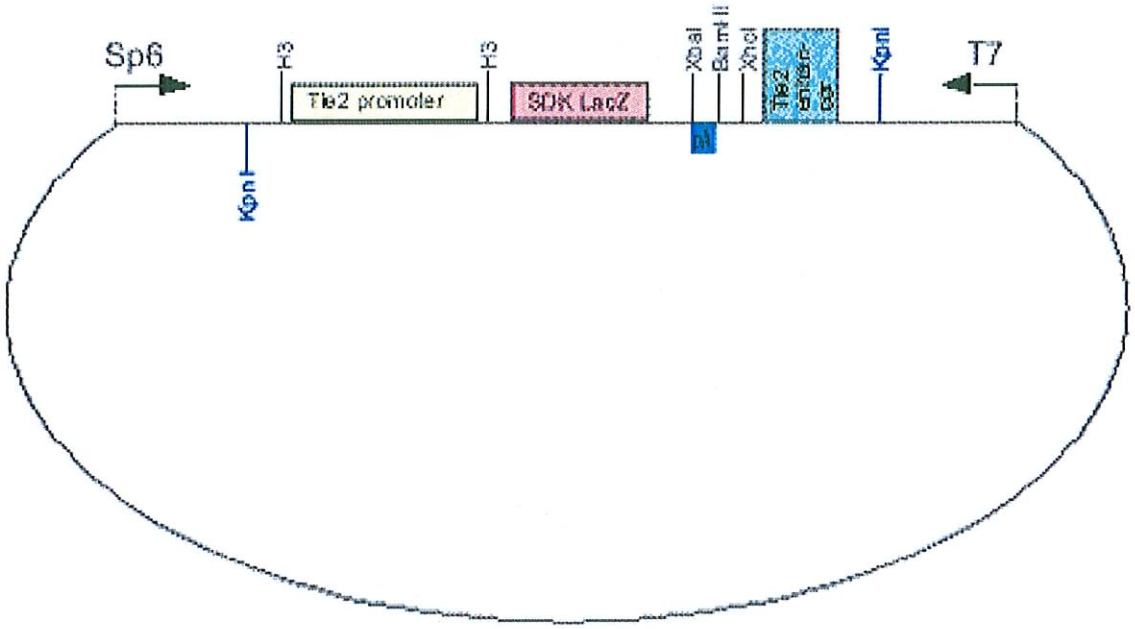
Name: pg50-2.11 (#65)

Parental Vector: pBSKII(+)

DNA Insert: Murine Tie2 5' promoter -1st exon-1st intron (~17kb) inserted into the Not I sit e of the vector

Antibiotic resistance: Amp

Produced by: Tom Sato



Name: pHHSDKXK (#19)

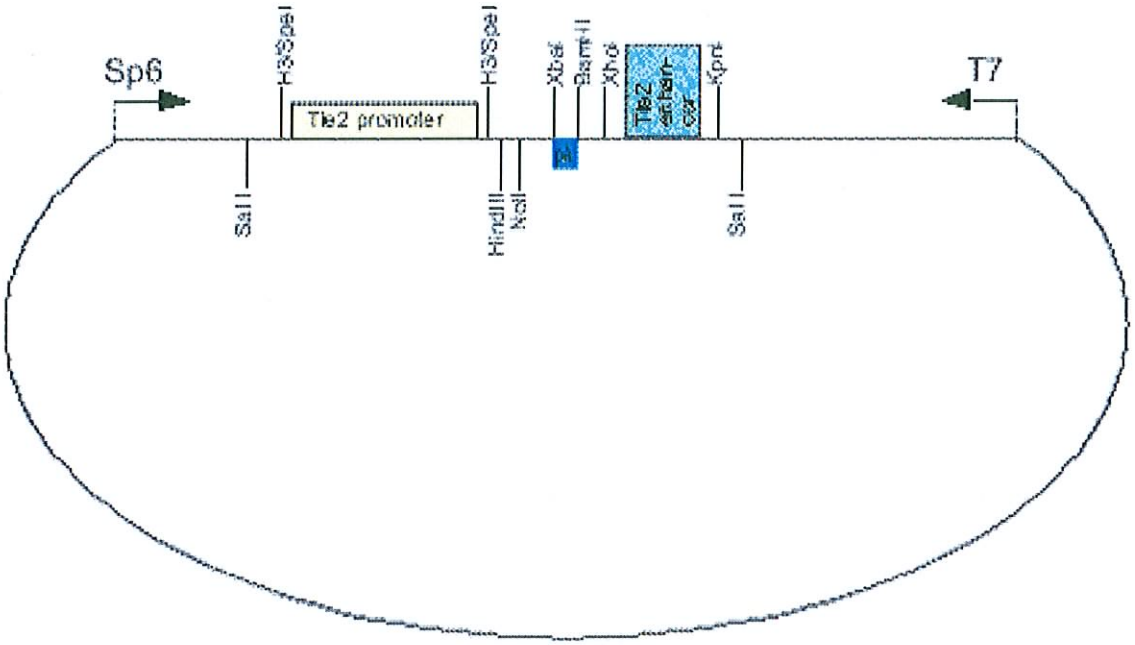
Parental Vector: pBS

DNA Insert: Tie2 promoter; 2kb (H3-H3) SDK LacZ; Tie2 enhancer (XhoI-KpnI)1.7kb

Antibiotic resistance: Amp

Produced by: Tom Sato

Additional Info and comments: Cut with KpnI to release the Tg fragment (~7kb)



Name: pSPTg.T2FpAXK (#52)

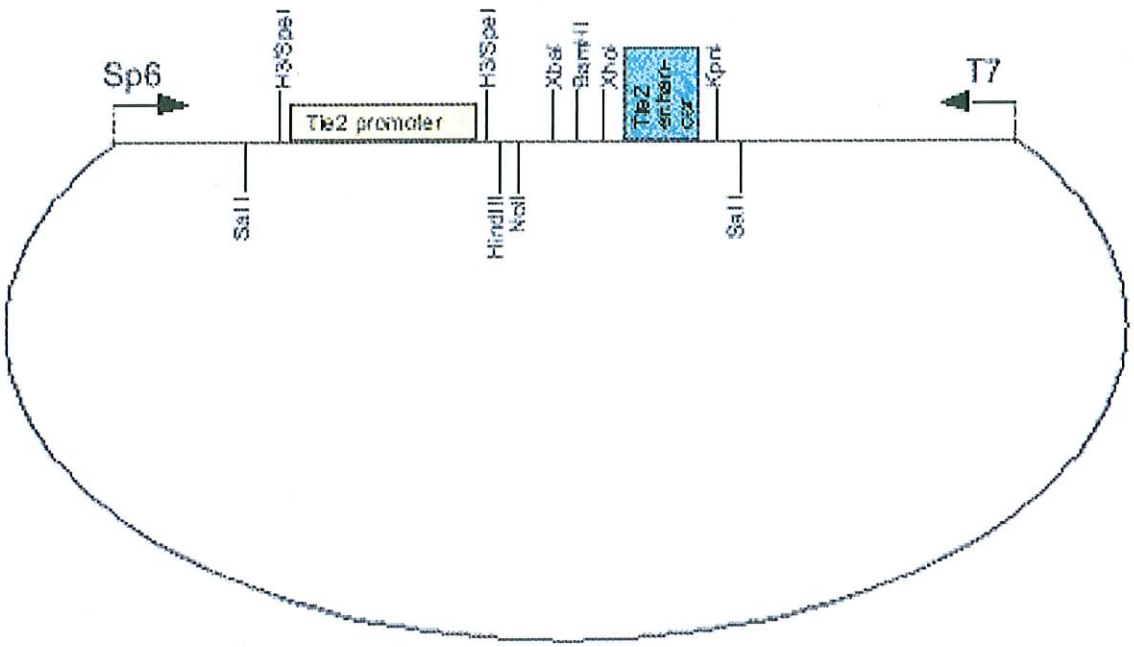
Parental Vector: pSPTg.F

DNA Insert: Tie2 promoter; 2kb PA; 0.3Kb Tie2 enhancer (XhoI-KpnI) 1.6kb

Antibiotic resistance: Amp

Produced by: Tom Sato

Additional Info and comments: The unique cloning sites are HindIII and Not I



Name: pSPTg.T2FXK (#54)

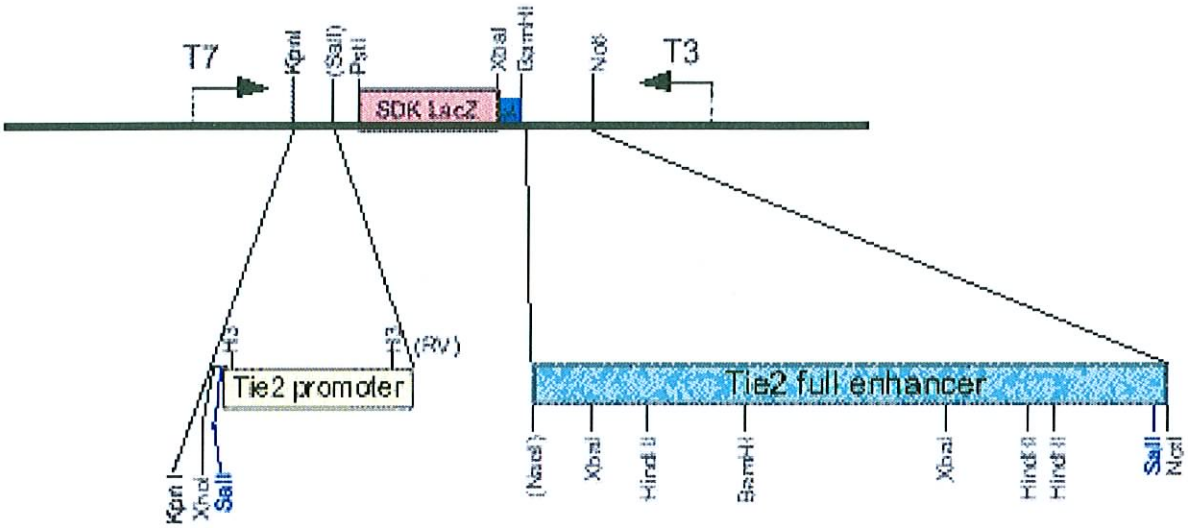
Parental Vector: pSPTg.F

DNA Insert: Tie2 promoter; 2kb (H3-H3) Tie2 enhancer (XhoI-KpnI) 1.6kb

Antiblotic resistance: Amp

Produced by: Tom Sato

Additional Info and comments: The unique cloning sites are HindIII and Not I



Name: pT2HLacZpA11.7 (#59)

Parental Vector: pBluescript SKII

DNA Insert: Tie2 (H2-H3) promoter - SDKLacZ-pA-Tie2 full enhancer (~15kb)

Antiblotic resistance: Amp

Produced by: Tom Sato

Additional Info and comments: Tg insert can be excised by Sal I digest (~15kb)

Summary of Approvals for Permit BIO-RRI-0028

Permit Holder: J.G. Pickering

Approved Personnel (Please stroke out any personnel to be removed

Theo Small
~~Dinath Ratnayake~~
Caroline Oneil
Zengxuan Nong
~~Cindy Ho~~
Matt Frontini
Nica Borradaile
~~Nicole Barbosa~~

Additional Personnel

Oula Akawi
Faran Vafaie

	Please stroke out any approved Biohazards* to be removed below	Write additional Biohazards for approval below.
Approved Microorganisms*	E.coli	
Approved Cells*	Human (established) HEK 293	→ Human Skin Fibroblast → Transformed human skin Fibroblast (Ataxia-telangiectasia) → Human Aortic Endothelial Cells
Approved Use of Human Source Material*		
Approved GMO*	Adeno E1A (HEK 293), Admax (Adeno), vectors- pD3111, pDC411, pDC511	→ pLNCX chick src Y527F plasmid → pcDNA3 Flag FKHR AAA mutant plasmid (FOXO1A)
Approved use of Animals*	Rats	

Date of last Biohazardous Agents Registry Form Jan 25, 2007

Signature of Permit Holder: J. Pickering

BioSafety Officer(s): J Stanley May 26/08

Biosafety Committee, Chair G.M. Kidder 26 May '08

2007-02

robarts RESEARCH

BIO-RRI-0026

BIOHAZARDOUS AGENTS REGISTRY FORM

Reviewed by Biosafety Subcommittee: February 2006

This form must be completed by each Principal Investigator when completing a grant application or grant renewal to be administered by the Robarts Research Institute, if the use of biohazardous and/or infectious agents is proposed. For any proposed animal work involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans, this form must also be completed.

COMPLETED FORMS ARE TO BE RETURNED TO BIOSAFETY SUBCOMMITTEE CHAIR, ROOM 3-34.1.

If there are any changes to the information on these forms (excluding grant title and funding agencies) a new form must be completed and sent to the Biosafety Subcommittee Chair BEFORE implementation of these changes can occur.

If multi-team grants are being applied for, each individual Investigator of the team must submit a Biohazardous Agents Registry Form to the Biosafety Subcommittee Chair.

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

For questions regarding this form, please contact Biosafety Subcommittee Chair at ext. 34125.

1.0 Contact Information

PRINCIPAL INVESTIGATOR: Dr. J.G. Pickering

SIGNATURE: J.G. Pickering

DATE: Jan. 15, 2007

DEPARTMENT: Vascular Biology

ADDRESS: Robarts Research Institute, 100 Perth Drive, London, ON, N6A 5K8

TELEPHONE: (519)

EMAIL: gpickering@robarts.ca

Location of experimental work to be carried out:

	Tissue culture work:	Animal work:
Building(s):	Robarts	Medical Science Building
Room(s):	E4-13	Health Sciences Animal Facility

**For work being performed at Institutions affiliated with the Robarts Research Institute, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Robarts Research Institute, Biosafety Subcommittee Chair. See Section 13.0, Approvals*

RECEIVED Jan. 19/07 EB

GRANT TITLE(S): Smooth Muscle Cells and Vascular Disease

ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) EXPLAINING THE BIOHAZARD(S) USED.

FUNDING AGENCY/AGENCIES: CIHR and HSFO

Anticipated Grant End Date: _____

Names of all personnel working under Principal Investigator's supervision in this location:

Nicole Barbosa
Nica Borridaile
Matt Frontini
Cindy Ho
Zengxuan Nong
Caroline O'Neil
Dinath Ratnayake
Theo Small
Eric van der Veer

Note : A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site : http://www.phac-aspc.gc.ca/ols-bsl/index.html

2.0 Microorganisms

2.1 Does your work involve the use of microorganisms? If **NO**, please proceed to Section **3.0**

YES ☑ NO ☐

2.2 Please complete the table below:

what e. coli used to make plasmids in 5.5-??

DH5α

Name of Microorganism	Is microorganism a known human pathogen? **YES/NO**	Is microorganism a known animal pathogen? **YES/NO**	Is microorganism a known zoonotic agent? **YES/NO**	Maximum quantity to be cultured at one time?	Health Canada or CFIA Containment Level (select one)
					1☐ 2☐ 3☐
					1☐ 2☐ 3☐
					1☐ 2☐ 3☐

3.0 Cell Culture

3.1 Does your work involve the use of cell cultures? YES ☑ NO ○
If **NO**, please proceed to Section **4.0**.

3.2 Please indicate in the table below the type of cells that will be grown in culture.

Cell Type	Is this cell type used in your work? **YES / NO**	Established or Primary *	Supplier of Primary Cell Culture Tissue
Human	Yes	Established	
Rodent	No		
Non-human primate	No		
Other (specify)			

* i.e. derived from fresh tissue

3.3 Complete the following table.

Specific Cell Line	Source / Supplier	HC or CFIA Containment Level (select one)
HEK 293	ATCC	1 ○ 2 ☑ 3 ○
		1 ○ 2 ○ 3 ○
		1 ○ 2 ○ 3 ○

4.0 Use of Human Source Materials

4.1 Does your work involve the use of human source materials? YES ○ NO ☑
If **NO**, please proceed to Section **5.0**

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

Human Source Material	Specify Source, or Not Applicable (NA)	Is Human Source Material known to be infected with an infectious agent? YES/NO	Name of Infectious Agent	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid				1○ 2○ 3○
Human Blood (fraction) or other Body Fluid				1○ 2○ 3○
Human Organs (unpreserved)				1○ 2○ 3○
Human Tissues (unpreserved)				1○ 2○ 3○

5.0 Genetically Modified Organisms and Cell lines

5.1 Will genetic modifications be made to the organism, virus or cell line? YES ☑ NO ○
If NO, please proceed to Section **6.0**

5.2 Will genetic sequences from any of the following be involved?

- HIV YES ○ NO ☑

If YES, specify: _____

- HTLV 1 or 2 YES ○ NO ☑

If YES, specify: _____

- Other human or animal pathogen and/or their toxins YES ○ NO ☑

If YES, specify: _____

5.3 Will intact genetic sequences be used from:

- SV 40 Large T antigen YES ○ NO ☑
- Adeno E1A YES ☑ (HEK293) BD NO ~~☑~~
- Known or suspected oncogenes YES ○ NO ☑

If YES, specify: Adeno E1A (HEK293).

5.4 Will a live vector(s) (viral or bacterial) be used for gene transduction? YES ☑ NO ○
If YES, name vector: Admax (Adenovirus type 5) — specific description + source — See attachment

5.5 List specific vector(s) to be used: pDC311, pDC411, pDC511

5.6 Will vector be replication defective? YES ☑ NO ~~☑~~ unless replication competent ??

5.7 Will vector be infectious to humans or animals? YES ☑ NO ○

5.8 Will this be expected to increase the Containment Level required? YES ○ NO ☑

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials using the vector(s) in 5.5 be conducted? YES ○ NO ☑
If NO, please proceed to Section **7.0**
If YES, attach a full description of the make-up of the virus.

6.2 Will vector be able to replicate in the host? YES ○ NO ○

6.3 How will the vector be administered? _____

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

_____

6.5 Has human ethics approval been obtained? YES ○ NO ○

Approval # _____

7.0 Animal Experiments

7.1 Will any of the agents listed be used in live animals? YES ☑ NO ○
If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used: Rats

7.3 AUS protocol # 2006-064-08

7.4 If using murine cell lines, have they been tested for murine pathogens? YES ○ N/A NO ☑

8.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs YES ○ NO ☑
- Pound source cats YES ○ NO ☑
- Sheep or goats YES ○ NO ☑
- Non- Human Primates YES ○ NO ☑

If **YES** specify species _____

- Wild caught animals YES ○ NO ☑

If **YES** specify species _____

9.0 Biological Toxins

9.1 Will toxins of biological origin be used? YES ○ NO ☑
If **NO**, please proceed to Section **10.0**
If **YES**, please name the toxin _____

9.2 What is the LD_{50} (specify species) of the toxin? _____

10.0 Import Requirements

10.1 Will the agent be imported? YES ○ NO ☑
If **NO**, please proceed to Section **11.0**
If **YES**, country of origin _____

10.2 Has an Import Permit been obtained from HC for human pathogens? YES ○ NO ○

10.3 Has an import permit been obtained from CFIA for animal pathogens? YES ○ NO ○

10.4 Has the import permit been sent to Biosafety Subcommittee Chair? YES ○ NO ○

If **YES**, Permit # _____

11.0 Training Requirements for Personnel Named on Form

All personnel named in section **1.0** of this form who will be using any of the above named agents are required to attend the following training courses given by OH&S.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 2.0 to 10.0 have been trained as required.

SIGNATURE _____

12.0 Containment Levels

12.1 For the work described in sections **2.0** to **10.0**, select the highest HC or CFIA Containment Level required. 1○ 2◉ 3○

12.2 Has the facility been certified by Biosafety Subcommittee Chair for this level of containment?

YES ◉ NO ○

If **YES**, give date: August 18, 2006 and permit number: 2006-08-(KRI)

13.0 Approvals

Robarts Research Institute

Signature _____ Date January 25, 2006

Biosafety Officer for the Institution where experiments will take place

Signature _____ Date _____

Biosafety Officer of Robarts Research Institute (if different than above)

Signature _____ Date _____

Note: This permit will be in effect from _____ to _____,

subject to annual facility re-certification.