

Modification Form for Permit BIO-UWO-0010

Permit Holder: Jeff Dixon

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

Dr. Souzan Armstrong
Mathew Grol
~~Matthew Sangpintho~~
Elizabeth Pruski
Dr. Alexy Pereverzev

Additional Personnel
(Please list additional personnel here)

Kim Beaucage (Grad Student)
Karen Ann Dodge (Grad Student)
Dr. Juan Pablo Reyes Valverde (PDF)

Please stroke out any approved Biohazards to be removed below

Write additional Biohazards for approval below. *

Approved Microorganisms

E. coli DH1-OB, DB 3.1, Adenovirus

Approved Cells

Rodent (primary), Human (established), HEK-293, Rodent (established), UMR 106, NIH, ROS, CFK2, NC3T3, RAW, Eahy 926, MC3T3 E1, CHO, Non-human primate (established), cos-1, cos-7

Clonetics Normal Human Osteoblasts
Genetics Human Osteoblast Precursors

Approved Use of Human Source Material

Approved GMO

SV 40 Large T antigen, cos-1, cos-7, Adenovirus, pKS-TK-NeoLoxP, YCplac22, IRES beta geo, pPGKCrebpA, pCEP-Pu-EBV, Nr.6 (ApaI-ClaI filling in), pcDNA3.1-Pu, Nr.20 (Pu from pCre-Pac, Sall digest.

Approved use of Animals

* 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: Feb 8, 2008

Signature of Permit Holder: *Jeff Dixon*

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Modification Form for Permit BIO-UWO-0010

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Approved Toxin(s)

Exoenzyme C3, Pertussis

[Empty box for additional toxins]

*Cells will be used for in vitro assays only.
Cultures will be handled at Biosafety Level 2.
See attached information sheets.*

* 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: Feb 8, 2008

Signature of Permit Holder:  (S.J. Dixon)

BioSafety Officer(s): _____

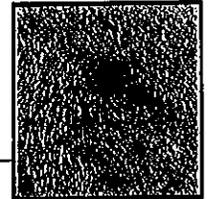
Chair, Biohazards Subcommittee: _____

Lonza

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Clonetics® Normal Human Osteoblast Cell System

NHOst



Introduction

Clonetics® Osteoblast Cell System contains Normal Human Osteoblasts (NHOst) and optimized medium for their growth. Each system can quickly generate NHOst cultures for experimental applications in bone research, physiology, cellular function and differentiation. Clonetics® Osteoblast Cell System is convenient and easy to use, allowing the researcher to focus on results. Cryopreserved NHOst are shipped in second or third passage. Proliferating NHOst are shipped in third or fourth passage.

Clonetics® Cells, Medium and Reagents are quality tested together and guaranteed to give optimum performance as a complete Cell System.

Cell System Components

- One Normal Human Osteoblast Cell Product (Cryopreserved or Proliferating)
- One Normal Human Osteoblast Cell Medium BulletKit® - 500 ml
 Clonetics® OGM™ BulletKit® (CC-3207) contains one 500 ml bottle of Osteoblast Basal Medium (OBM™) and OGM™ SingleQuotes® containing the following growth supplements: FBS, 50 ml; Ascorbic Acid, 0.5 ml; GA-1000, 0.5 ml.
- Differentiation SingleQuotes® (CC-4194)
 Hydrocortisone 21 Hemisuccinate (CC-4333), 0.5 ml (For alkaline phosphatase up-regulation and bone mineralization). β-Glycerophosphate (CC-4399), 5 ml (1.0 M solution - for bone mineralization)
- One ReagentPack™ (CC-5034) Containing:

Trypsin/EDTA	100 ml
Trypsin Neutralizing Solution	100 ml
HEPES Buffered Saline Solution	100 ml

Characterization of Cells

Routine characterization of NHOst includes immunofluorescent staining. NHOst test positive for alkaline phosphatase and bone mineralization.

Performance

Recommended Seeding Density	5,000 cells/cm ²
Typical time from subculture to confluent monolayer	5 - 9 days
Cumulative population doublings guaranteed using Clonetics® System	10

Quality Control

All cells are performance assayed and test negative for HIV-1, mycoplasma, Hepatitis-B, Hepatitis-C, bacteria, yeast and fungi. Cell viability, morphology and proliferative capacity are measured after recovery from cryopreservation. Clonetics® Media are optimized to grow specific types of normal human cells. Each lot of medium is tested for the support of cell viability and proliferative capacity. Certificates of Analysis (CoA) for each cell strain are shipped with each order. CoA for all other products are available upon request.

Ordering Information

Cryopreserved Cells

CC-2538 NHOst ≥500,000 cells

Proliferating Cells – Flasks and Multiwell Plates

CC-2538T25 T-25 Flask

CC-2538T75 T-75 Flask

CC-2538W96 96-well Plate

Other proliferating formats are available. Contact Technical Service or refer to the Lonza website for details.

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CC-3207	OGM™ BulletKit®, OBM™ plus SingleQuots® of growth supplements	500 ml
CC-3208	OBM™, Osteoblast Basal Medium	500 ml
CC-4193	OGM™ SingleQuots®, Formulates OBM™ to OGM™	
CC-4194	OGM™ Differentiation SingleQuots® induce osteoblast differentiation and mineralization	
CC-5034	ReagentPack™	
	Trypsin/EDTA Solution	100 ml
	Trypsin Neutralizing Solution	100 ml
	HEPES Buffered Saline Solution	100 ml

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza guarantees the performance of its cells only if Clonetics® Media and Reagents are used exclusively, and the recommend protocols are followed. The performance of cells is not guaranteed if any modifications are made to the complete Cell System. Cryopreserved NHOst are assured to be viable and functional when thawed and maintained properly.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-I, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, Biosafety in Microbiological and Biomedical Laboratories, 1999. If you require further information, please contact your site Safety Officer or Technical Services.

note



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Document # TS-AA-1034-6 03/08
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Poietics® Osteoclast Precursor Cell System

OCP



Introduction

Poietics® Osteoclast Precursor Cell System contains normal Human Osteoclast Precursors (OCP) and differentiation medium that can be set up in a 96 well format to conduct research on osteoporosis, bone resorption, osteoporosis or other bone related diseases. Osteoclasts are large multinucleated cells that play an active role in bone resorption. Monocytes from the marrow or the blood serve as precursors of osteoclasts. Currently researchers drive monocytes to become osteoclasts with less than 10% efficiency. In contrast our Osteoclast Precursor Cell System yields up to 50% conversion of precursors to osteoclasts when differentiated. The cell system is convenient and easy to use, allowing the researcher to focus on results. OCP are shipped frozen.

Poietics® Cells, Medium and Reagents are quality tested together and guaranteed to give optimum performance as a complete Cell System. Each Cell System can be custom designed to meet any specific research need. List individual catalog numbers for cells and medium when ordering.

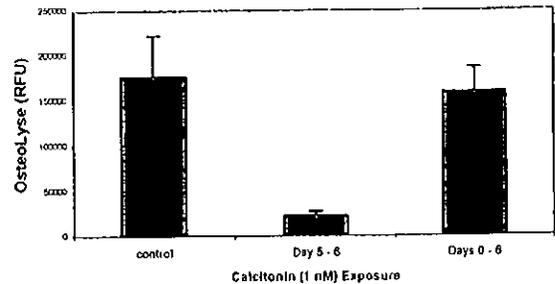
Cell System Components (Need to be purchased separately)

- One Osteoclast Precursor Cell Product (Cryopreserved)
- One Osteoclast Precursor Cell Medium Product (BulletKit®)- 100 ml
OPGM™ BulletKit® (PT-8001) contains one 100 ml bottle of OPBM Basal Medium and the following differentiation supplements: soluble RANK ligand, 0.1 ml; M-CSF, 0.1 ml; L-glutamine, 1ml; FBS, 10 ml; Pen/Strep, 1 ml.

Characterization of Cells

OCP are characterized morphologically at day 7 as large, multinucleated cells. Differentiated cells stain positive for tartrate-resistant acid phosphatase. Additional characterization assays are available upon request for an additional fee.

Effects of Calcitonin on Osteoclast-Mediated Bone Matrix Degradation In Vitro



Inhibition of bone matrix resorption by calcitonin as assayed in the OsteoLyse™ Assay. Primary Human Osteoclast Precursors were seeded onto an OsteoLyse™ Plate at 10,000 cells/well and cultured in differentiation medium containing no calcitonin, calcitonin added only at day 5 and calcitonin added on days 0 and 5. Ten µl samples of supernatants were counted after a total of 6 days. Calcitonin, added at day 0, resulted in the osteoclasts becoming refractory to calcitonin added on day 5.

Performance

Recommended seeding density for subculture	30,000 cells/cm ²
	96 well plate-
	10,000 cells per well

Typical time from seeding to differentiated monolayer 7 days

**These cells do not undergo population doublings and will senesce in the absence of specific differentiation signals.

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Quality Control

Cell viability and morphology are measured after recovery from cryopreservation. Poietics® Media are formulated for optimal growth of specific types of normal human cells. Each lot of medium is tested for the support of cell viability and differentiation capacity. Certificates of Analysis (CA) for each cell strain are shipped with each order. CA for all other products are available upon request. Non-routine performance and quality testing to meet your specifications is available for an additional fee.

Ordering Information

2T-110	OCP Osteoclast Precursors	1,000,000 cells/amp
PT-8001	OCGM BulletKit®	
PT-8201	OCBM Osteoclast Precursor Basal Medium	100 ml
PT-9501	OCGM SingleQuots® Soluble RANK Ligand M-CSF L-glutamine FBS Pen/Strep	

When placing an order or for technical service, please refer to the product numbers and descriptions listed above. For a complete listing of all Clonetics® Products, refer to the Lonza website or the current Lonza catalog. To obtain a catalog, additional information or technical service you may contact Lonza by web, e-mail, telephone, fax or mail.

Product Warranty

CULTURES HAVE A FINITE LIFESPAN IN VITRO. Lonza warrants its cells only if Poietics® Media are used, and the recommended protocols are followed. Cryopreserved OCP cells are assured to be viable and functional when thawed and maintained properly.

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro procedures.

WARNING: CLONETICS® AND POIETICS® PRODUCTS CONTAIN HUMAN SOURCE MATERIAL, TREAT AS POTENTIALLY INFECTIOUS. Each donor is tested and found non-reactive by an FDA approved method for the presence of HIV-1, Hepatitis B Virus and Hepatitis C Virus. Where donor testing is not possible, cell products are tested for the presence of viral nucleic acid from HIV, Hepatitis B Virus, and Hepatitis C Virus. Testing can not offer complete assurance that HIV-1, Hepatitis B Virus, and Hepatitis C Virus are absent. All human sourced products should be handled at the Biological Safety Level 2 to minimize exposure of potentially infectious products, as recommended in the CDC-NIH Manual, [Biosafety in Microbiological and Biomedical Laboratories](#), 1999. If you require further information, please contact your site Safety Officer or Technical Services.

**THE UNIVERSITY OF WESTERN ONTARIO
 BIOHAZARDOUS AGENTS REGISTRY FORM
 Revised Biohazards Subcommittee: January, 2007**

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr. Jeff Dixon
 SIGNATURE 
 DEPARTMENT Dentistry and Physiology & Pharmacology
 ADDRESS R-0075 LG DSB
 PHONE NUMBER 661 3769
 EMAIL Jeff.Dixon@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 0078 0078A

*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
① Ion transport and signaling in skeletal cells: P2 nucleotide receptor function in bone, ② Regulation of bone resorption: control of ion channels and calcium in osteoclasts.

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES CIHR

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

- i) Elizabeth Pruski
- ii) Alexey Peterverzev
- iii) Mathew Grol
- iv) Souzan Armstrong
- v) Nattapon Panupinthu
- Pastor Solano

1.0 Microorganisms

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

1.2 Please complete the table below:

Name of Biological agent(s)	Is it known to be a human pathogen? YES/NO <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Is it known to be an animal pathogen? YES/NO <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Is it known to be a zoonotic agent? YES/NO <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Maximum quantity to be cultured at one time?
<i>E. Coli</i> DB3.1	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	1 Litre
Adenovirus	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Five T-75 Flasks
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	
	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	<input type="checkbox"/> Yes <input type="checkbox"/> No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required.

1 2 3

1.4 Source of microorganism(s) or biological agent(s)?

ATCC & INVITROGEN

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 (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work? <input type="checkbox"/> Yes <input type="checkbox"/> No	Source of Primary Cell Culture Tissue
Human	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	mouse, rat
Non-human primate	<input type="checkbox"/> Yes <input type="checkbox"/> No	
Other (specify)		

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

Cell Type	Is this cell type used in your work? <input type="checkbox"/> Yes <input type="checkbox"/> No	Specific cell line(s)	Supplier / Source
Human	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	HEK-293	INVITROGEN
Rodent	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	UMR 106, NIH, ROS	
Non-human primate	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	CFR 2, NC3T-3	
Other (specify)	<input type="checkbox"/> Yes <input type="checkbox"/> No	RAW, EAhy 926 MC3T3E1, CHO	

2.4 For above named cell types(s) circle HC or CFIA containment level required 1 2 3

cos-1, cos-7

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

DB 3.1
DB 3.1

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO

3.2 Indicate if the following will be used in the laboratory
Human blood (whole) or other bodily fluids YES NO If YES, Specify
Human blood (fraction) or other bodily fluids YES NO If YES, Specify
Human organs (unpreserved) YES NO If YES, Specify
Human tissues (unpreserved) YES NO If YES, Specify

3.3 Is human source known to be infected with and infectious agent YES NO
If YES, please name infectious agent N/A

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

4.0 Genetically Modified Organisms and Cell lines

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

4.2 Will genetic sequences from the following be involved:
HIV YES NO
if YES specify
HTLV 1 or 2 or genes from any CDC class 1 pathogens YES NO
if YES specify
Other human or animal pathogen and or their toxins YES NO
if YES specify

4.3 Will intact genetic sequences be used from
SV 40 Large T antigen YES NO If YES specify COS-1, cos-7
Known oncogenes YES NO If YES specify

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction YES NO
If YES name virus Adenovirus

4.5 List specific vector(s) to be used: SEE ATTACHED LIST.

4.6 Will virus be replication defective YES NO

4.7 Will virus be infectious to humans or animals YES NO

4.8 Will this be expected to increase the Containment Level required YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

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

5.3 How will the virus be administered? _____

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

5.5 Has human ethics approval been obtained? YES NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? YES NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 If using murine cell lines, have they been tested for murine pathogens? YES NO

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other 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 _____
colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin Exoenzyme A3 & Pertussis *See attached*

8.3 What is the LD₅₀ (specify species) of the toxin Not available

Requirements: Toxins must be locked at all times.
Keep less than 5mg on hand. Use less than 1 human ~~toxic~~ lethal dose if possible / known.

9.0 Import Requirements

9.1 Will the agent be imported?

ف YES ~~ف NO~~

If no, please proceed to Section 10.0

If yes, country of origin _____

9.2 Has an Import Permit been obtained from HC for human pathogens? ف YES ف NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ف YES ف NO

9.4 Has the import permit been sent to OHS? ف YES ف NO

If yes, Permit # _____

10.0 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

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

SIGNATURE 

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please circle the highest HC or CFIA Containment Level required. 1 (2) 3

11.2 Has the facility been certified by OHS for this level of containment? ~~ف YES~~ ف NO

11.3 If yes, please give the date and permit number: _____

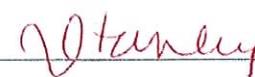
12.0 Approvals

UWO Biohazard Subcommittee

Signature 

Date 8 Feb. '08

Safety Officer for Institution where experiments will take place

Signature 

Date Feb 7/08

Safety Officer for University of Western Ontario (if different than above)

Signature _____

Date _____

Vectors. Store at -20°C . Alex: Room 0031, 81361.

1. pKS-TK-NeoLoxP
2. YCplac22
3. IRES β geo
4. pPGKCrebpA
5. pCEP-Pu-EBV, Nr.6 (ApaI-ClaI filling in)
6. pcDNA3.1-Pu, Nr.20 (Pu from pCre-Pac, SalI digest; Hugro PvuII deletion)
7. pCAGGS-Flpe-IRES-Puro
8. pBKS
9. pEGFP-1
10. pREP7
11. pGC2-FRT2Neo
12. pCEP4
13. pCEP-Pu
14. pSPT18
15. pSPT19
16. pBSK
17. pEGFP-C1
18. pcDNA3.1/Hygro(+)
19. pAdVantage
20. pABWN
21. pCre-Pac
22. pcDNA3
23. pSL1180
24. pECFP-1
25. pPKC α -EGFP
26. pCAGGS-GFP
27. pEBFP-N1
28. pEYFP-1
29. pIRES-hrGFP-1a
30. pGT1.8IRES β Geo
31. pCAGGS-GFP-Neo (Neo ClaI-SalI fragment from pHM2 inserted into SalI)
32. pHM2
33. pBST-SK
34. pRAY-1
35. pTeT off
36. pBI-EGFP
37. pHcRed1-1
38. pDsRed1-1
39. pIRES
40. pIRES2-EGFP (pIG)
41. pIRES2-EGFP without promoter (pIG-Pr.)
42. pCEP-Pu-LIF58
43. pGEX2T-LIF58
44. pIRES2-DsRed2
45. pIRESneo3
46. pEYFP-N1