Dr. M. Prado

Modification Form for Permit BIO-RRI-0052

Permit Holder: Marco Prado

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

Jue Fan
Sanda Raulic
Jillian Roberts
Monica Guzman
Flavio Paiva
Fabiana Caetano
Xavier De Jaeger
Iaa Numes
Ivana Souza

Additional Personnel
(Please list additional personnel here)

Amro Mohamad
Ashbeel Roy
Valeriy Ostapchenko
Amanda Martyn
Zoltan Kiss
Andre Guimaraes
Juan Carlos

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. Give the full name - do not abbreviate.
Approved Microorganisms	E.coli, Yeast, AAV serotype 2	
Approved Primary and Established Cells	Established: Human: HEK-293. Rodent: CF10, SN56, L929	mouse neurons, astro cytes and cardiomyocites
Approved Use of Human Source Material		
Approved Genetic Modifications (Plasmids/Vectors)	Adeno associated viral vector, pEGFP, pCMV-myc, pCMV-HA, pGEX, pACT2, pEGFP, pGADT7, pGBKT7, pMAL, pPICZ	adenovirus-cre vector replication deficient provided by Sean Cregan iZEG plasmid
Approved Use of Animals	Mus Musculus	
Approved Biological Toxin(s)	Spider Venom Toxins (phoneutria nigriventer) Fraction 3	

* *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 STORED, USED AND DISPOSED OF..*

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: _____

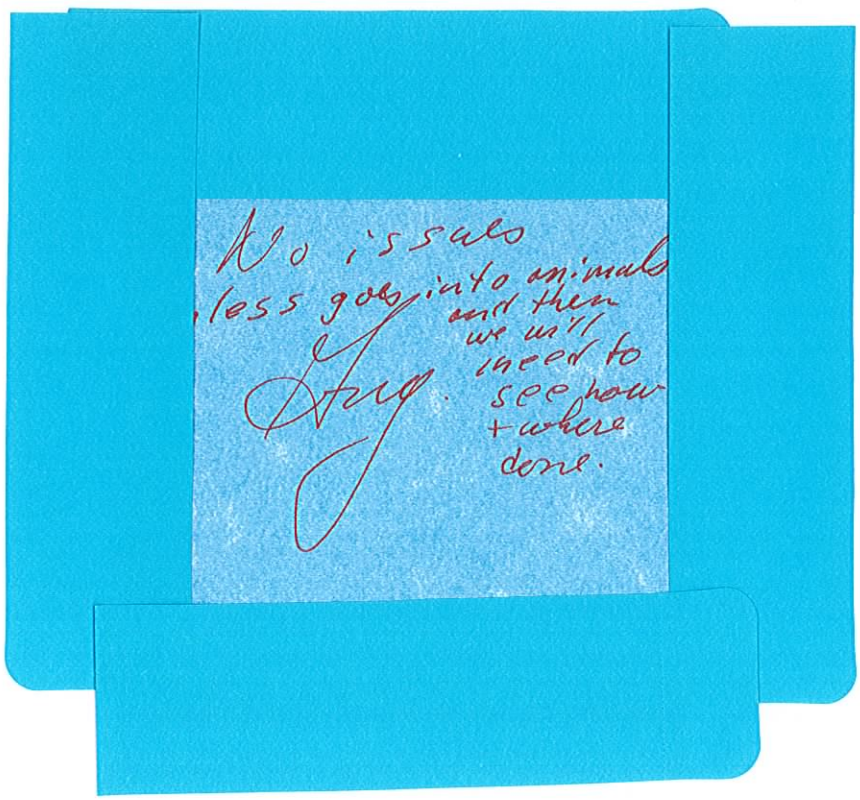
Current Classification: 2 **Containment Level for Added Biohazards:** _____

Date of Last Biohazardous Agents Registry Form: Mar 13, 2009

Date of Last Modification (if applicable): Sep 9, 2009

BioSafety Officer(s): Ronald Deslauriers Feb 17, 2011

Chair, Biohazards Subcommittee: _____ **Date:** _____



Ron Noseworthy

From: Marco Prado [mprado@robarts.ca]
Sent: Tuesday, February 08, 2011 5:30 PM
To: Jennifer Stanley; 'rsn@uwo.ca'
Subject: RE: FW: prionet
Attachments: Prado_February_2011_MOD.pdf

Thanks. There are some change in people and I will also add another construct. We will obtain an adenovirus construct (replication deficient) expressing Cre and GFP from Sean Cregan. The adenovirus will be produced and purified by Robert Gros in his lab, which he has been doing for a number of people at Robarts, we will not produced it in our lab. This adenovirus expressing Cre is not new, it has been used regularly by others..

We will use it to infect primary cultured cells for now. We may in the future infect cardiac tissue in mice. If we decide to perform these experiments in mice we will do with Ian Welch in a level 2 lab. There was no space in the form for all this explanations.

Jennifer please let me know if I can provide you with further information. We are aware that the adenovirus should be used only in the level 2 lab.

Thanks

Marco

Dr. Marco A.M. Prado, Ph.D.
Scientist, Molecular Brain Research Group
Robarts Research Institute
Professor,
Department of Physiology & Pharmacology and
Department of Anatomy & Cell Biology
The University of Western Ontario
P.O. Box 5015, 100 Perth Drive
London, ON N6A 5K8
Tel: 519-663-5777 Ext. 24888
Fax: 519-663-3789
mprado@robarts.ca
www.robarts.ca www.uwo.ca

robarts
MOLECULAR BRAIN RESEARCH

From: Jennifer Stanley [mailto:jstanle2@uwo.ca]
Sent: February-08-11 5:07 PM
To: 'rsn@uwo.ca'; Marco Prado
Subject: Fwd: FW: prionet

Hi there
Here it is. along with the instructions.
Thanks for sending along the plasmid map.
Regards
Jennifer

-------- Original Message --------
Subject:FW: prionet
Date: Tue, 08 Feb 2011 12:44:39 -0500
From: Ron Noseworthy <rnoseworthy@robarts.ca>
To:jstanle2@uwo.ca <jstanle2@uwo.ca>
CC: Marco Prado <mprado@robarts.ca>

Hi Jennifer,

Can you send Dr. Prado a Biohazard modification form?

Thanks

Ron

-----Original Message-----
From: Marco Prado
Sent: Tuesday, February 08, 2011 12:33 PM
To: Ron Noseworthy
Subject: FW: prionet

Hi Ron

I will start to use this plasmid. Can you help me and add it to my biosafety protocol

Thanks

Marco

Dr. Marco A.M. Prado, Ph.D.
Scientist, Molecular Brain Research Group
Robarts Research Institute
Professor,
Department of Physiology & Pharmacology and
Department of Anatomy & Cell Biology
The University of Western Ontario
P.O. Box 5015, 100 Perth Drive
London, ON N6A 5K8
Tel: 519-663-5777 Ext. 24888
Fax: 519-663-3789
mprado@robarts.ca
www.robarts.ca www.uwo.ca

-----Original Message-----
From: jirik@ucalgary.ca [mailto:jirik@ucalgary.ca]
Sent: February-02-11 3:51 PM
To: Marco Prado
Cc: mlcoulte@ucalgary.ca
Subject: RE: prionet

Marco-here is the map and the sequence of the vector we have used.
f

i2eg

CLONE RECORD

Clone Name: pCCALL2-IRES-EGFP/anton **Date:** 22. Jan. 03.

Vector: _____ **Vector Supplier:** Dr. Connie Lobe

Insert size: _____ **Total Size:** 10985 bp

Sites cloned into: _____

Originally Obtained from: _____

Bacteria: DH5α **Antibiotic resistance:** Amp

Read sense from (orientation): _____

Sequence confirmation: yes no (please circle)

Sequence disk attached: yes no (please circle)

Map attached: (yes) no (please circle)

Promoters: Chicken beta-actin

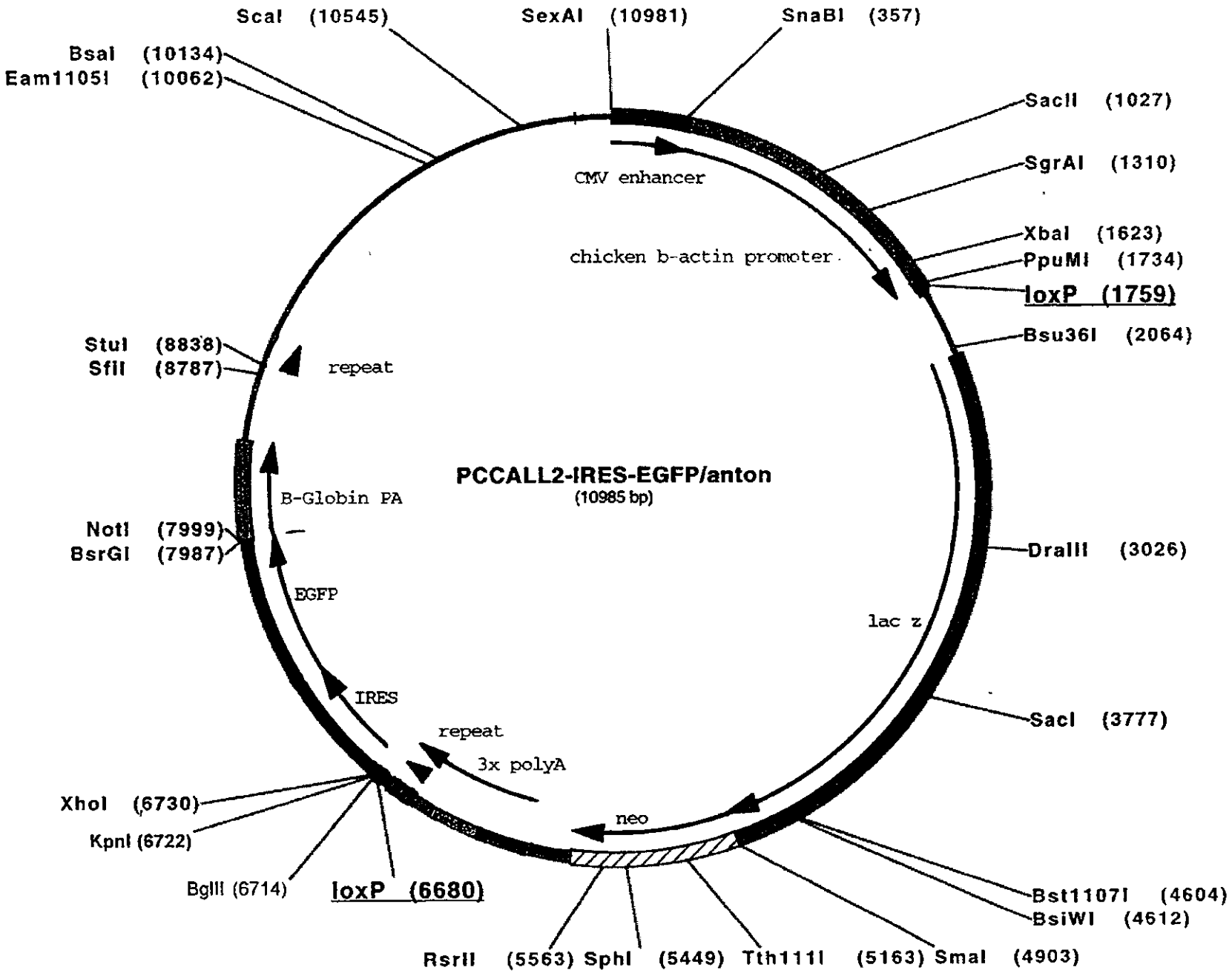
Glycerol stocks location: Lab stocks box

DNA location: _____

Aliquot in lab stock box: (yes) no (please circle)

Person who made clone: Glycerol stocks + plasmid (Artee)

Comments: _____


ScaI (10545)
SexAI (10981)
SnaBI (357)
BsaI (10134)
Eam1105I (10062)
SacII (1027)
SgrAI (1310)
CMV enhancer
chicken b-actin promoter
XbaI (1623)
PpuMI (1734)
loxP (1759)
Bsu36I (2064)
StuI (8838)
SfiI (8787)
repeat
PCCALL2-IRES-EGFP/anton
(10985 bp)
B-Globin PA
NotI (7999)
BsrGI (7987)
DraIII (3026)
EGFP
lac z
IRES
SacI (3777)
repeat
3x polyA
XhoI (6730)
KpnI (6722)
neo
BglII (6714)
loxP (6680)
Bst1107I (4604)
BsiWI (4612)
RsrII (5563)
SphI (5449)
Tth111I (5163)
SmaI (4903)

Modification Form for Permit BIO-RRI-0052

Permit Holder: Marco Prado

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

Fabiana Caetano

Xavier De Jaeger

Iaa Numes -- IACI NUNES

Ivana Souza

~~Cristina Martins Silva~~

~~Cristiane Menezes~~

~~Rita Pires~~

Additional Personnel
(Please list additional personnel here)

Flavio Henrique Bonaldo di Paiva

MONICA GUZMAN

JILLIAN Roberts

SANDA RAULIC

JUE FAN

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E.coli, Yeast, AAV serotype 2	
Approved Cells	Established: Human: HEK-293. Rodent: CF10, SN56	L929
Approved Use of Human Source Material		
Approved GMO	Adeno associated viral vector, pEGFP, pCMV-myc, pCMV-HA, pGEX, pACT2, pEGFP, pGADT7, pGBKT7, pMAL, pPICZ	
Approved use of Animals	Mus Musculus	
Approved Toxin(s)	Spider Venom Toxins (phoneutria nigriventer) Fraction 3	

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** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.

As the principal investigator, I have ensured that all of the personnel named on the form have been trained. I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca.

Signature of Permit Holder: [signature]

Classification: 2

Date of Last Biohazardous Agents Registry Form: Mar 13, 2009

BioSafety Officer(s): [signature] Sept 9/09

Chair, Biohazards Subcommittee: [signature]

These cells will be used in the project Physiological function of the Prion Protein to study the trafficking of the cellular prion protein.

L929 cell line

These cells will be used in the project Physiological function of The Prion Protein to study the trafficking of the cellular prion protein.

ATCC
The Global Bioresource Center

Search Catalog
Select a Category
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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 [illegible] 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 [illegible] for pricing information and to place an order for ATCC cultures and products.

Cell Biology

ATCC® Number:	**CCL-1™** Order this Item	**Price:**	**$256.00**
Designations:	NCTC clone 929 [L cell, L-929, derivative of Strain L]		**Related Links**
Depositors:	WR Earle		
	1		
Shipped:	frozen		
Medium & Serum:			
Growth Properties:	adherent		
Organism:	*Mus musculus* (mouse)		
Morphology:	fibroblast		
Source:	**Tissue:** subcutaneous connective tissue; areolar and adipose **Strain:** C3H/An		
Permits/Forms:	In addition to the ITA mentioned above, other ATCC [illegible] regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [illegible] for information regarding the specific requirements for shipment to your location.		
Isolation:	**Isolation date:** March, 1948		
Applications:	testing [illegible] toxicity testing [illegible] transfection host (toxicology [illegible] [illegible]		
Virus Susceptibility:	vesicular stomatitis (Indiana); encephalomyocarditis		
Virus Resistance:	poliovirus 1, 2, 3; coxsackievirus B5; polyomavirus		
Tumorigenic:	Yes		
Reverse Transcript:	positive		
Antigen Expression:	H-2k		
Cytogenetic Analysis:	modal chromosome number = 66; range = 65 to 68. There were approximately 20 to 30 marker chromosomes present in each metaphase spread. A high percentage of those markers were common to most analyzed cells. A long metacentric chromosome with secondary constriction was noted in 77/100 cells.		
Age:	100 days		
Gender:	male		

Comments: NCTC clone 929 (Connective tissue, mouse) Clone of strain L was derived in March, 1948. Strain L was one of the first cell strains to be established in continuous culture, and clone 929 was the first cloned strain developed. The parent L strain was derived from normal subcutaneous areolar and adipose tissue of a 100-day-old male C3H/An mouse. [25276]
Clone 929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain. [13964]
Tested and found negative for ectromelia virus (mousepox).

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: horse 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 1:2 to 1:8 is recommended
Medium Renewal: 2 to 3 times per week

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

Related Products: derivative:ATCC CCL-1.1
derivative:ATCC [illegible]
derivative:ATCC [illegible]
derivative:ATCC [illegible]
Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC [illegible]

Bioreactive Factors: **Growth Factors:** T cell growth factor (TCGF)

References:

3: Kazazian HH Jr., et al. Restriction site polymorphism in the phosphoglycerate kinase gene on the X chromosome. Hum. Genet. 66: 217-219, 1984. PubMed: 6325224
21373: Fisher EM, et al. Homologous ribosomal protein genes on the human X and Y chromosomes: escape from X inactivation and possible implications for Turner syndrome. Cell 63: 1205-1218, 1990. PubMed: 2124517
21404: Sanford KK, et al. The growth in vitro of single isolated tissue cells. J. Natl. Cancer Inst. 9: 229-246, 1948.
21405: Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943-945, 1985. PubMed: 3933111
21469: ASTM International Standard Practice for Direct Contact Cell Culture Evaluation of Materials for Medical Devices. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 0813-07.
21470: ASTM International Standard Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity. West Conshohocken, PA:ASTM International;ASTM Standard Test Method F 0895-84 (Reapproved 2006).
21606: U.S. Pharmacopeia General Chapters: <87> BIOLOGICAL REACTIVITY TESTS, IN VITRO. Rockville, MD:U.S. Pharmacopeia;USP USP28-NF23, 2005
23579: Westfall BB, et al. The glycogen content of cell suspensions prepared from massive tissue culture: comparison of cells derived from mouse connective tissue and mouse liver. J. Natl. Cancer Inst. 14: 655-664, 1953. PubMed: 13032849
25770: Earle WR, et al. Production of malignancy in vitro. IV. The mouse fibroblast cultures and changes seen in the living cells. J. Natl. Cancer Inst. 4: 165-212, 1943.
25879: Earle WR, et al. The influence of inoculum size on proliferation in tissue cultures. J. Natl. Cancer Inst. 12: 133-153, 1951. PubMed: 14874126
25880: Sanford KK, et al. The tumor-producing capacity of strain L mouse cells after 10 years in vitro. Cancer Res. 16: 162-166, 1956. PubMed: 13295695
25882: Westfall BB, et al. The arginase and rhodanese activities of certain cell strains after long cultivation in vitro. J. Biophys. Biochem. Cytol. 4: 567-570, 1958. PubMed: 13587550
29223: Papkoff J. Regulation of complexed and free catenin pools by distinct mechanisms. J. Biol. Chem. 272: 4536-4543, 1997. PubMed: 9020160
32283: Hu SX, et al. Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression. Cancer Res. 57: 3339-3343, 1997. PubMed: 9269991
33114: Yasin B, et al. Susceptibility of Chlamydia trachomatis to protegrins and defensins. Infect. Immun. 64: 709-713, 1996. PubMed: 8641770
92346: Biological evaluation of medical devices. Part 5: Tests for in vitro cytotoxicity. Sydney, NSW, Australia:Standards Australia;Standards Australia AS ISO 10993.5-2002.
92380: Plastics collapsible containers for human blood and blood components--Part 1: Conventional containers. Annex C. Biological tests.. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 3826-1:2003.
92382: Dentistry--Preclinical evaluation of biocompatibility of medical devices used in dentistry--Test methods for dental materials. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 7405:1997.
92389: Biological evaluation of medical devices--Part 5: Tests for in vitro cytotoxicity. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 10993-5:1999.
92404: Plastic containers for intravenous injection. Geneva (Switzerland):International Organization for Standardization/ANSI;ISO ISO 15747:2003.

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THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: April, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents are 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. This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be 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, OHS (Stevenson-Lawson Building, Room 295) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR MARCO ANTONIO M. PRADO
SIGNATURE
DEPARTMENT Physiology/Pharmacol + Anatomy/Cell Bio
ADDRESS Robarts Institute R3-105
PHONE NUMBER 685 8500 X36888
EMAIL MPRADO@ROBARTS.CA

Location of experimental work to be carried out: Building(s) ROBARTS INST. Room(s) 3-07 v1 3-07 v2

*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 Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: CIHR / NIH / FAPESP
GRANT TITLE(S): - The role of basal forebrain cholinergic neurons in synaptic plasticity and cognition
- Genetic Analysis of cholinergic function
- Physiological Function of Prion Protein II

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

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

- Rita Pires
- Cristiane Menezes
- Cristina Martins Silva
- Ivana de Souza
- Ilca Vieira
- Xavier De Jaeger
- Fabiana Caetano

(#1) AAV-UE and AAV-GFP are based on AAV serotype 2. The viral vectors are going to be produced by Dr. Robert Greene from University of Texas, USA, at a final titer of 1.3×10^{12}/ml and sent to us

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)? O YES O 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	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	Health Canada or CFIA Containment Level
E. coli	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	1 L	GE/UEB	O 1 O 2 O 3
yeast	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	1 L	Invitrogen/ Clontech	O 1 O 2 O 3
AAV serotype 2	O Yes ⊗ No	O Yes ⊗ No	O Yes ⊗ No	(#1)	Dr. Robert Greene	O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

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

2.0 Cell Culture

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

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

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

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

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No	HEK-293	ATCC
Rodent	O Yes O No	CFID ; SN56	Wilma Martins / Bruce Wainer
Non-human primate	O Yes O No		
Other (specify)	O Yes O 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 HC or CFIA containment level required O 1 ⊗ 2 O 3
HEK-293 CL2
CFID and SN56 CL1

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

3.0 Use of Human Source Materials

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

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	HC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 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 O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. coli	Several	several	several	N.A.

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

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

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results
ADENO ASSOCIATED VIRAL VECTOR		Dr. Robert Gagne	• Cre-recombinase • GFP	• Deletion of floxed allele • Fluorescent protein

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ ☒ NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ ☒ NO
- SV 40 Large T antigen O YES ☒ NO
- E1A oncogene (HEK 293) ☒ YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins ☒ YES, please specify _____ O NO
  (Ca calcium and potassium channel toxins will be expressed in bacteria or yeast)

4.5 Will virus be replication defective? ☒ YES O NO

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

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

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

5.0 Human Gene Therapy Trials

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

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

5.3 How will the virus be administered? _____

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

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

6.0 Animal Experiments

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

6.2 Name of animal species to be used Mus musculus

6.3 AUS protocol # 2008:089 (submitted)

6.4 Will any of the agents listed be used in live animals ☒ YES, specify: AAV-Cre AAV-GFP O NO

7.0 Use of Animal species with Zoonotic Hazards

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

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s) spider venom toxins (Phoneutua nigriventer) - Fraction 3
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin 137 ug/kg mouse

9.0 Import Requirements

9.1 Will the agent be imported? ☒ YES, please give country of origin USA O NO
If no, please proceed to Section 10.0

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

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

9.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ ☒ NO

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

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:

Not everybody arrived yet. As soon as they arrive they will be Trained.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS
- Employee Health and Safety Orientation

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

SIGNATURE _____

11.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O1 X2 O3 → completed Mar 11/09

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

O YES, permit # if on-campus_____
X NO
O NOT REQUIRED the lab. is being set up at the moment. As soon as it is ready to go we will ask for certification.

12.0 Procedures to be Followed

12.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories. I will ensure that workers have an up-to-date Position Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: August 12, 2008

13.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: S.M. Kidder
Date: 13 March 2009

Safety Officer for Institution where experiments will take place: SIGNATURE: Stanley
Date: March 12/09

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number BIO: ~~[illegible]~~ PR1-005 2 Expiry Date (3 years from Approval): March 13, 2012

Special Conditions of Approval:

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

Marco

Subject: 2008-089 Prado
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Tue, 30 Sep 2008 14:52:56 -0400
To: Kathy Floyd <kfloyd@uwo.ca>
CC: Ron Noseworthy <rnoseworthy@robarts.ca>, "Gregory A. Dekaban" <dekaban@robarts.ca>
BCC: "Gerald M. Kidder" <avpres@uwo.ca>

Kathy

This is approved with the statements:

1.6 - Level 2 containment post procedure unless PCR or other data shows that there is no viral shedding. N95 respirator to be worn for injection (contact Ashley Acott for respirator fit test). Blunt Hamilton syringes should be used. If possible, micropipettes should be not be re-used.

Other comments:

The Biohazard Subcommittee reviewed the Biohazardous Agents Registry Form on September 30, 2008. This work requires Level 2 containment post procedure. It "CAN BE considered Level i only when PCR or other data shows that they are not shedding virus".

Thanks,
Jennifer

-------- Original Message --------
Subject: 2008-089 Stanley
Date: Tue, 30 Sep 2008 14:05:53 -0400
From: Kathy Floyd <kfloyd@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

2008-089 Prado 08 19 08 Final VP.doc
Content-Type: APPLICATION/msword
Content-Encoding: base64

Vania F. Prado and Marco A. M. Prado

BRIEF DESCRIPTION THAT EXPLAINS BIOHAZARDS USED AND HOW THEY WILL BE USED

The role of basal forebrain cholinergic neurons in synaptic plasticity and cognition

Basal forebrain cholinergic neurons undergo moderate degenerative changes during normal aging and the resulting cholinergic hypofunction has been associated to age-related memory deficits.

Mutant mice with brain region selective vesicular acetylcholine transporter (VAChT) ablation are an important tool to allow the elucidation of cholinergic function in specific regions of the brain without the complex interpretations in behavior experiments that arise from disturbing the peripheral cholinergic system (such as neuromuscular deficits).

We have used genetic recombination to generate novel mouse models of dementia by targeting the vesicular acetylcholine transporter (VAChT). This protein is responsible for the accumulation of ACh into synaptic vesicles and is thought to mediate the rate-limiting step for ACh release. For this proposal, we have generated floxed VAChT mice (Flanked by loxP), to use the Cre/LoxP strategy to create mouse lines with brain region specific deletion of the VAChT gene. The enzyme Cre mediates a recombination event that removes loxP intervening sequences and therefore can remove the floxed VAChT gene. This gene targeting strategy allows temporal and spatial control of gene inactivation.

We will use the approach of infusing an adenovirus vector to drive the expression of Cre in the Nucleus basalis or medial septum of $VAChT^{flox/flox}$ mice to generate animals with ablation of VAChT from neurons that project either to the cortex or hippocampus respectively. We will also use the adenovirus vector to generate animals with ablation of VAChT from neurons that project to the heart and other tissues.

Using adenovirus vector expressing Cre to generate brain region specific VAChT KO. We will also rely on injecting the $VAChT^{flox/flox}$ mice with AAV-Cre construct that leads to Cre expression in neurons when injected stereotaxically in the brain [62]. The advantage of using such vectors is that the recombinant virus cannot replicate and therefore expression of Cre should be limited to the site of injection. Dr. Robert Greene from the University of Texas Southwestern will provide the AAV-Cre virus and we will infuse the virus to either the nucleus basalis or to the medial septum to knockout the VAChT gene in cholinergic neurons that project to the cortex or the hippocampus. To do that, mice will be anaesthetized and placed into a stereotaxic apparatus (Kopf). A single scalp incision is made. A burr hole (approximately 3 mm in diameter) is made in the skull and a glass micro pipette filled with 1 microliter of AAV-Cre is going to be stereotaxically microinjected into a specific brain area (into either the medial septum (0.98 AP, 0 LL and 3.25 DV) or into the nucleus basalis (-0.94 AP, 1.5 LL and 3.25 DV)). Only one area of the brain will be injected in each animal. To minimize tissue injury,these injections are

going to be performed using glass pipettes with a 10- to 20-micrometer diameter tip, and AAV-Cre is going to be slowly injected over 1 hr using a pressure-injection system. After an additional period of 5 minutes, the micropipette is removed and the scalp incision is closed with wound clips. Sham surgeries will be performed the same way, except that the pipette will be filled with vehicle, and no injection will be made. Animals are kept on heat pads or under heat lamps to maintain body temperature throughout the procedures. During recovery, animals are kept in the surgery room to allow frequent monitoring of breathing and general activity (according to SOP330-03 POST-OPERATIVE/POST ANAESTHETIC CARE RODENTS) before being transported back to the holding room. The procedure is going to be performed once in each animal and we will use adult mice (8-20 wks). Mice will be tested for VAChT immunofluorescence to evaluate the effectiveness of the gene deletion.

To verify whether we have generated inducible or gene specific VAChT KO mice, for each unique mouse strain produced we will confirm that the VAChT gene has been solely deleted from cholinergic neurons from the aimed area by immunofluorescence. We have used all these methods to characterize VAChT KD mice and will perform the experiments as described in Prado et al. (2006).

Increasing cholinergic neurotransmission

We identified two possible approaches that could be used to improve the release of ACh in transgenic mice. One is directed to the high affinity choline transporter (CHT1). In cholinergic neurons, high affinity uptake of choline is mediated by CHT1, a protein expressed predominantly in cholinergic neurons, that has been identified recently.

A second approach to generate mice that can release ACh more effectively will be to target the VAChT. A single point mutation (A228G) in a transmembrane segment of VAChT facilitates reorientation of the transporter across the vesicular membrane, easing a conformation restraint for fast activity; this mutant has over three-fold more activity than wild-type VAChT in vitro. Literature data suggest that increased expression of VAChT in cultured neurons increases ACh packing in synaptic vesicles. Because the VAChT mutant (A228G) increases Vmax for ACh transport we expect that *in vivo* the outcome will be similar (i.e. more ACh packing in vesicles).

Generate transgenic mice expressing the L531A mutant of CHT1 or the A228G mutant of VAChT. Considering that conventional transgenic constructs <20 Kb frequently lead to lack of expression or incorrect expression, we decided to prepare our transgenic constructs using BACs (bacterial artificial chromosome).

CHT1 construct: The CHT1 L531A transgenic construct is prepared using the mouse BAC RPN23-268K23, that contains the CHT1 gene, obtained from the BACPAC Resource Center, Children's Hospital Oakland Research Institute, Oakland, California. Using this BAC, we changed the L531 codon (CTA) present at the C-terminal end of the transporter to an alanine codon (GCT) and added an HA tag to the C-terminus (The HA tag should facilitate biochemical detection of transgenic CHT1, but does not affect transport activity *in vitro* (see Ribeiro et al., 2003, 2005). The "recombineering" system with the galK selection marker was used for generation of the mutated BAC. This

system allows for high-efficiency homologous recombination in *E. coli* and permits modification of BACs without restriction enzymes or DNA ligases.
VAChT construct: We obtained the mouse BAC RP24-108G1 containing the VAChT gene from BACPAC Resource Center and used a similar approach as described above to generate "super-VAChT" (A228G).

Prado V. F., et al. (2006) Mice deficient for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition. *Neuron* **51**, 601-612.

Provisional Patent Application: Animal model of cholinergic dysfunction to evaluate cognitive enhancers and drugs that improve myasthenia 5405-400PR -PRADO, M. A. M. ; CARON, Marc G ; PRADO, V F ; IZQUIERDO, I ; PEREIRA, G S ; CASTRO, B M ; SILVA, C M .

Mechanisms involved in the cellular trafficking of the high affinity choline transporter (CHT1) , the vesicular acetylcholine transporter (VAChT) and Cellular prion protein (PrPc).
We have been studying trafficking of the high affinity choline transporter (CHT1), the vesicular acetylcholine transporter (VAChT) and the cellular prion protein (PrPc) in culture cells (HEK-293, SN56, CF-10). To do that we transfect cells with fluorescent proteins (CHT1-WT tagged with HA, CHT1 mutants tagged with HA, EGFP-VAChT, EGFP-VAChT mutants, VAChT-HA, VAChT-HA mutants, GFP-PrPc, GFP-PrPc mutants) and follow the trafficking of the recombinant protein in live cells using confocal microscopy. Mutants that interfere with different trafficking pathways (mutants of rab5, rab7, rab11, AP180, dynamin-1) are also used in these studies.

Barbosa-Jr et al .*J. Neuroch.* 73, 1881 (1999).
Santos et al. *J. Neurochem* 78, 1104 (2001).
Barbosa-Jr et al. *J. Neurochem.* 82,1221 (2002);
Ribeiro et al. *J. Neurochem.* 87, 136 (2003).
Ribeiro et al. *J. Neurochem.* 94,86 (2005).
Ferreira et al. *J. Neurochem* 94, 957 (2005);
Ribeiro et al. *Eur. J. Neuroscience* 26, 3437 (2007).
Caetano FA et al. Endocytosis of prion protein is required for ERK1/2 signaling induced by stress-inducible protein 1.J Neurosci. 2008 28(26):6691-702.
Magalhães AC et al. Uptake and neuritic transport of scrapie prion protein coincident with infection of neuronal cells.J Neurosci. 2005 May 25;25(21):5207-16.
Prado MA et al. PrPc on the road: trafficking of the cellular prion protein.
J Neurochem. 2004 Feb;88(4):769-81.
Magalhães AC et al. Endocytic intermediates involved with the intracellular trafficking of a fluorescent cellular prion protein. J Biol Chem. 2002 Sep 6;277(36):33311-8.
Lee KS et al. Internalization of mammalian fluorescent cellular prion protein and N-terminal deletion mutants in living cells. J Neurochem. 2001 Oct;79(1):79-87.

Developing novel drugs based on peptides.
We have cloned, expressed and characterized different toxins isolated from the venom of the spider *Phoneutria nigriventer*. These peptides are known to interfere with the function of different ion channels and therefore are important tools to help in the understanding of basic functions of these channels. Furthermore, they can be potentially used as drugs with important medical applications. We are currently developing approaches to use these recombinant peptides as painkillers and anti-arrhythmic drugs.

Souza et al. Analgesic effect in rodents of native and recombinant Phα1 toxin, a N-type calcium channel blocker isolated from armed spider venom. Pain. In Press (2008)
Carneiro et al. *Toxicon 41, 305* (2003);
Matavel et al. *FEBS Letters* 523, 219 (2002);
Penaforte et al. *Toxicon* 38, 1443 (2000);
Kushmerick et al. *J. Neurochem* 72,1472 (1999);
Guatimosim et al *Toxicon* 37, 507 (1999);
Kalapothakis et al. *Toxicon* 36, 1971 (1998);
Kalapothakis et al. *Toxicon* 36, 1843 (1998).

Provisional Patent Application: Toxin Phalpha1Beta, cDNA of toxin Phalpha1Beta, pharmaceutical preparations of Phalpha1beta, manufacturing, and generation of cDNA and product. PI0605484-6 GOMEZ, M. V. ; PRADO, M. A. M., PRADO V.F. et al.

Proposal for standard operating procedure for use of Recombinant Viral Vectors for intracranial injections in rodents.

In neuroscience, there is increasing need to study the involvement of particular genes in specific neurological disease or behaviour. An elegant approach to address such questions is to study the effects of manipulations of gene expression in restricted areas of the brain. The most commonly used method is the use of recombinant viral vectors to express a particular gene, via injections into the brain area of interest. This technique has now been used for over a decade at universities all over the world (including Canada) and has yielded breakthrough findings important for human health and disease.

The viral vectors most commonly used include: Adenovirus, Adeno-associated virus (AAV), Herpesvirus, and Lentivirus:

The purpose of this communication is to begin establishing a *standard operating procedure for all investigators at UWO* that want to use viral vectors by injections into the brain. mMany researchers at UWO are eager to be able to perform these experiments to greatly advance their research, as there is a great need for such standard operating procedures. It is our understanding that protocols for the use of lentivirus are currently being established. However, use of adenovirus and adeno-associated virus is rarely subject to the same safety measures as the use of lentivirus, thus we propose a separate protocol for the use of these particular viralvectors.

The goal of this proposed standard operating procedure is to describe handling of the vector immediately prior to injections in the animals, procedures during surgery, maintenance of animals following injections, and disposal of viral vector and animals.

The information in this document was generated in consultation with researchers at other universities in Ontario, universities in other provinces in Canada, as well as in the United States of America and Europe. In addition, Drs. Sean Cregan, Vania Prado, Marco Prado, and Lique Coolen already have experience with the use of adenovirus and adeno-associated virus and protocols associated with these vectors, during previous tenures at universities in Canada, USA, and Brazil.

Proposed procedures for Adenovirus and Adeno-associated virus:
Adenovirus and adeno-associated virus are replication defective or non-replicating and are most commonly handled at Biosafety Level 2 (BL2). Adeno-associated virus in the absence of a helper virus" can in most cases be handled at Biosafety Level 1.

Pre-surgery handling of viral vectors: Hamilton syringes or glass micropipettes used for the surgeries are filled in a ~~fume hood~~ laminar flow hood and transported to the surgery facility in a closed containe:

Surgery: All small animal surgery space is subject to BL 2 regulations: Personnel is required to wear appropriate protective gear, consisting of cap, Gown, mask, goggles, and gloves. These requirements already apply to all rodent surgeries. All standard surgery

procedures for intracranial injections are followed. None of these surgeries require use of fume hoods or biosafety cabinets.

Disposal of viral vectors and micropipettes: instruments and needles are soaked in bleach solution prior to disposal. Animal carcasses do not need special treatment.
Post-operative care: Standard operating procedures for rodent surgeries will be followed; no special treatment of the animals is required.

Suggested contact person:
These protocols are well established and have been used by numerous investigators at the University of Ottawa. For more information, please contact:
Dr. Jennifer Keyte DVM CVAv
Veterinary Officer Animal Care and Veterinary Service, University of Ottawa.
tel: (613) 562-5800 x8071
fax: (613) 562-5467

Plasmid list:

- pEGFP (Clontech)
- pCMV-myc (Clontech)
- pCMV-HA (Clontech)
- pGEX (GE)
- pACT2 (Clontech)
- pEGFP (Clontech)
- pGADT7 (Clontech)
- pGBKT7 (Clontech)
- pMAL (NEB)
- pPICZ (Invitrogen)