SIMS

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

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

Mao Jiang

Souzan Armstrong

Tom Chrones

Juan Reyes-Valverde

Ben Wheal

Ryann Shugg

Alexey Pereverzev

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, Recombinant Adenovirus	
Approved Cells	Human (primary), colon esophagus, Rodent (primary), Human (established) HEK 293, Rodent (established) RAW 264.7, CHo, Non-human primate (established) COS,	
Approved Use of Human Source Material	tissues (unpreserved), esophagus, colon	
Approved GMO	SV 40 Large T antigen, COS, Adenovirus, pcDNA3, pEGFP, pE4FP, pAd/CMV/VS-DEST, plasmid EGFP-LC3, pCEP4YPet-MAMM (yPET)	Plasmid: pcDNA3.1-P2x2-YFP
Approved use of Animals	rabbits, guinea pigs, rats mice	
Approved Toxin(s)	Tetrodo toxin, ~~Botulinum toxin~~, Pertussis toxin	

* *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.*

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: _____

Classification: 2

Date of Last Biohazardous Agents Registry Form: Nov 22, 2007

Date of Last Modification (if applicable): Oct 26, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

- Jan. 20 2010
- Explanation for use of P2X2-YFP
- This plasmid encodes an ion channel (referred to as P2X2) which is labelled with a fluorescent probe.
- We will express the plasmid in cells and observe the location of the channel.

Regards. Stephen Smi

addgene

Find this plasmid at: www.addgene.org
Enter "22400" in the search box

Plasmid 22400: pcDNA3.1-P2X2-YFP

Gene/insert name:	ATP-gated P2X channel 2
Alternative names:	P2X2 receptors
Insert size (bp):	2300
GenBank/Entrez ID of insert:	NM_053656
Gene/insert aliases:	P2rx2, P2X2
Species of gene(s):	R. norvegicus (rat)
Fusion proteins or tags:	YFP
Terminal:	C terminal on backbone
Vector backbone:	pcDNA3.1 (Search Vector Database)
Backbone manufacturer:	Invitrogen
Type of vector:	Mammalian expression
Backbone size (bp):	5600
Cloning site 5':	HINDIII
Site destroyed during cloning:	No
Cloning site 3':	XHOI
Site destroyed during cloning:	No
5' Sequencing primer:	T7 (List of Sequencing Primers)
3' Sequencing primer:	BGH-Rev
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Selectable markers:	Neomycin
If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid:	James Fisher and Ali Jones in Cambridge
Sequence:	Visit www.addgene.org/22400
Plasmid Provided In:	DH5a
Principal Investigator:	Henry A. Lester

Article: An angstrom scale interaction between plasma membrane ATP-gated P2X2 and alpha4beta2 nicotinic channels measured with fluorescence resonance energy transfer and total internal reflection fluorescence microscopy. Khakh BS et al. (J Neurosci. 2005 Jul 20. 25(29):6911-20. Pubmed)

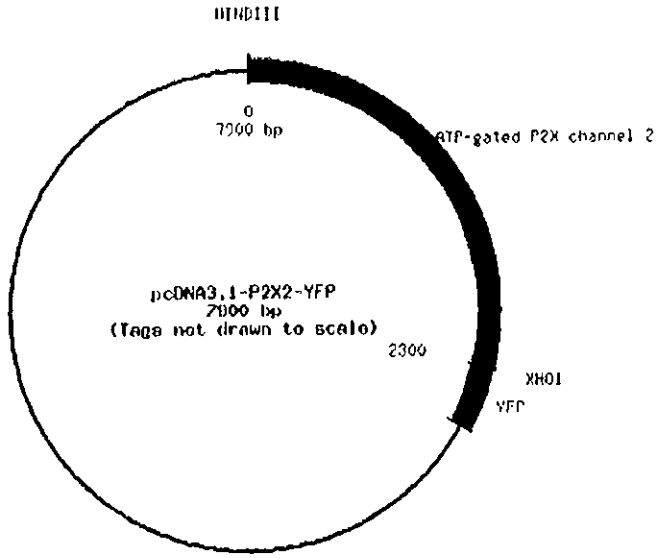
Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 22400" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication.

Please check www.addgene.org/22400 for updated plasmid information and related links.

addgene

Find this plasmid at: www.addgene.org
Enter "22400" in the search box



Please check www.addgene.org/22400 for updated plasmid information and related links.

Modification Form for Permit BIO (UWO)-00096

Permit Holders Stephen Sims

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

~~Danielle Duplaine~~

Mao Jiang

Souzan Armstrong

Tom Chrones

Additional Personnel
(Please list additional personnel here)

- Alexey Pereverzev
- Ryann Shuggs
- Ben Wheal
- Juan Pablo Reyes-Valverde

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Approved Use of Human Source Material	tissues (unpreserved), esophagus, colon	
Approved GMO	SV 40 Large T antigen, COS, Adenovirus, pcDNA3, pEGFP, pE4FP, pAd/CMV/V5-DEST, plasmid EGFP-LC3	plasmid: pCEP4YPet-MAMM YPET.
Approved use of Animals	rabbits, guinea pigs, rats mice	
Approved Toxin(s)	Tetrodo toxin, ~~Botulinum toxin~~, Pertussis toxin	

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
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Signature of Permit Holder: Stephen Sim Oct 14 2009

Classification: 2

Date of Last Biohazardous Agents Registry Form: Nov 22, 2007

Date of Last Modification (if applicable): Mar 27, 2009

BioSafety Officer(s): Oct 26/09

Chair, Biohazards Subcommittee: C.M. Kidder

Oct 14 2009

- We wish to purchase the YPET plasmid from a reputable organization.

- YPET is a fluorescent biomarker similar to Green fluorescent protein (GFP) that has enhanced fluorescence properties. Accordingly, we wish to use this to attach to protein of interest for expression in mammalian cells.

- The YPET fluorescence will then be monitored using confocal microscopy.

- These procedures are all worked out in the lab for GFP and will just use the new marker.

Regards. Stephen Sims

addgene

Find this plasmid at: www.addgene.org
Enter "14032" in the search box

Plasmid 14032: pCEP4YPet-MAMM

Gene/insert name:	YPet
Insert size (bp):	Unknown
Species of gene(s):	Other
Relevant mutations/deletions:	Mammalian optimized YPet
Vector backbone:	pCEP4 (Search Vector Database)
Type of vector:	Mammalian expression
Backbone size (bp):	10410
Cloning site 5':	Unknown
Site destroyed during cloning:	Unknown
Cloning site 3':	Unknown
Site destroyed during cloning:	Unknown
5' Sequencing primer:	See map (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
Sequence:	Visit www.addgene.org/14032
Plasmid Provided In:	DH5a
Principal Investigator:	Patrick Daugherty

Comments: The assembled sequence for this plasmid is inaccurate. The plasmid is actually 2.3kb smaller than the sequence suggests.

Article: Evolutionary optimization of fluorescent proteins for intracellular FRET. Nguyen AW et al. (Nat Biotechnol. 2005 Mar . 23(3):355-60. Pubmed)

Please acknowledge the principal investigator and cite this article if you use this plasmid in a publication.

Also, please include the text "Addgene plasmid 14032" in your Materials and Methods section. This information allows Addgene to create a link from the plasmid page to your publication

addgene

Find this plasmid at: www.addgene.org
Enter "14032" in the search box

Please check www.addgene.org/14032 for updated plasmid information and related links.

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

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

Danielle Lapierre

Mao Jiang

Souzan Armstrong

Tom Chrones

Additional Personnel
(Please list additional personnel here)

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Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder Stephen Sims Feb 17 2009

BioSafety Officer(s): March 27/09

Chair,Biohazards Subcommittee:

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

Approved Toxin(s) Tetrodo toxin, Botulinum toxin, Pertussis toxin

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Classification: 2

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder Stephen Sims Feb 17 2009

BioSafety Officer(s): [illegible] March 27/09

Chair,Biohazards Subcommittee: C.M. Kelder

1st. We will receive the plasmid (which is a small strand of DNA that encodes for the production of a specific protein PLUS the Green Fluorescent Protein (GFP) marker) and will replicate it using bacterial culture.
2nd. After enough of the plasmid has been replicated we will use it to tranfect bone cells (osteoclast-like and osteoblasts in culture). Tranfection pretty much involves the addition of the plasmid to the cells in culture in order for the plasmid DNA to enter the cells and replicate. The GFP tag allows us to monitor the protein expression.

That is all.
Let me know if you need more detailed information.
Thanks,
Tom

Modification Form for Permit BIO-UWO-0096

Permit Holder: Stephen Sims

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

Danielle Lapierre

Mao Jiang

Souzan Armstrong

Tom Chrones

Additional Personnel
(Please list additional personnel here)

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

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder: Stephen Sims May 26 2008

BioSafety Officer(s): J Stanley June 27/08

Chair, Biohazards Subcommittee: G.M. Kidder 2 July '08

Modification Form for Permit BIO UWO 0096

Permit Holder: Stephen Sims

Approved Toxin(s) Tetrodo toxin, Botulinum toxin ~~Pertussis toxin~~

- Pertussis toxin is used to study cell signalling pathways.
- The toxin targets a specific G protein, which is involved in signalling for ~~by~~ certain receptors.
- We purchased the smallest amount available, and will handle it only in the biosafety cabinet.
- 25 mm culture dishes containing cells will be treated with pertussis toxin and maintained in a culture incubator for 4 hours.
- Coverslips with treated cells will be removed from the incubator, washed, and viewed on a microscope.
- Residual solution containing pertussis toxin will be mixed with bleach, in accordance with existing practices.

* *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.*

Date of last Biohazardous Agents Registry Form: Nov 22, 2007

Signature of Permit Holder: Stephen Sims May 26 2008

BioSafety Officer(s): Gen Stanley June 23/08

Chair, Biohazards Subcommittee: G. M. Elder 2 July 08

Bio-UWO-0016

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 Stephen M. Singy
SIGNATURE Stephen Sin
DEPARTMENT Physiology & Pharmacology
ADDRESS DSB 0073 UWO
PHONE NUMBER 661-3768
EMAIL stephen.sing@schulich.uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 0071
*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): Please See Attached

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

Names of all personnel working under Principal Investigators supervision in this location.
i) Tom Chrones
ii) Mao Jiang
iii) Jourdan Armstrong
iv) Danielle Lapierre
v) Jason Kovac

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

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	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?
E. Coli	☒ Yes ☐ No	☒ Yes ☐ No	☒ Yes ☐ No	1500 mL
Recombinant Adenovirus non-replicating	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	2 x T75 Flasks
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ 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 microrganism(s) or biological agent(s)? 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?	Source of Primary Cell Culture Tissue
Human	☒ Yes ☐ No	Colon, Esophagus, obtained from surgical specimens
Rodent	☒ Yes ☐ No	Rats (Mice (Bone and Bone marrow) Rat (penis tissue)
Non-human primate	☐ Yes ☒ 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?	Specific cell line(s)	Supplier / Source
Human	☒ Yes ☐ No	HEK 293	Invitrogen, ATCC
Rodent	☒ Yes ☐ No	RAW 264.7, C2C12	ATCC
Non-human primate	☒ Yes ☐ No	COS	ATCC
Other (specify)	☐ Yes ☐ No		

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

* 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? ☒YES ☐NO
If no, please proceed to Section 4.0

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 Esophagus, colon

3.3 Is human source known to be infected with and infectious agent ☐YES ☒NO
If YES , please name infectious agent _____

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 CDS
- 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, non-replicating derivatives of

4.5 List specific vector(s) to be used: pcDNA3, pEGFP, pEYFP, pAd/CMV/V5-DEST (Invitrogen)

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

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 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 Rabbits, guinea pigs → @ AUS protocol #.

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 Tetrodotoxin & Botulinum Toxin

8.3 What is the LD_{50} (specify species) of the toxin
Tetrodotoxin LD50 for Humans is 5-30 mg/kg
Botulinum Toxin for Humans is 3 ng/kg
✓ Must keep less than 5 mg of each toxin at
✓ Must store in locked fridge/freezer at

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

9.0 Import Requirements

9.1 Will the agent be imported? ☐ YES ☒ 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? ☐ YES ☐ NO

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

9.4 Has the import permit been sent to OHS? ☐ YES ☐ 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 _____ Oct 17 2007

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? ☒ YES ☐ NO

11.3 If yes, please give the date and permit number: B10-UWO-0096 01

12.0 Approvals

UWO Biohazard Subcommittee

Signature _____ Date 22 Nov. 07

Safety Officer for Institution where experiments will take place

Signature _____ Date Nov 22/07

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

Signature _____ Date _____

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