Modification Form for Permit BIO-UWO-0008

Permit Holder: Linda Pagotto

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

~~Amanda MacGillivery~~

Lylia Nini

David Judah

Ernest Ho

Timothy Irvine

Randeep Singh

Kerry-Ann Nakrieko

Additional Personnel
(Please list additional personnel here)

ALENA RUDKOUSKAYA

LINDA VI

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	E. Coli DH5 alpha	
Approved Cells	Human (primary), rodent (primary), human (established) HeLa, HEK 293, Rodent (established) NIH-3T3 fibroblasts. HEK 293Tcells, MEL-5 melanoma cells	
Approved Use of Human Source Material		
Approved GMO	Adenovirus, mPD2.G, psPAX2	PCAG-CRE:GFP
Approved use of Animals		
Approved Toxin(s)	Cholera 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: Sep 24, 2007

Date of Last Modification (if applicable): Jan 30, 2009

BioSafety Officer(s): _____

Chair, Biohazards Subcommittee: _____

Jennifer Stanley
Biosafety Officer
Occupational Health & Safety
Support Services Building
Room 4190C

Tames Irvine
Research Technician
Dagnino Lab
Department of Physiology & Pharmacology
Seibens-Drake Research Institute
Room 230

RE: Modification Form for Permit BIO-UWO-0008

Jennifer,

Dr. Dagnino would like to add the plasmid CAG-Cre:GFP to the list of approved Biohazards on our BioSafety permit (BIO-UWO-008). The plasmid will be used to transfect primary murine keratinocytes for the sole purpose of expressing Cre recombinase within said cells. The expression of Cre recombinase results in a Cre-mediated recombination event and the deletion of a gene of interest when flanked by loxP sites.

Cheers,

Tames
X81372

addgene

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pCAG-Cre:GFP
Plasmid 13776

Browse > Connie Cepko > Matsuda et al > pCAG-Cre:GFP

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Price: $65.00

Plasmid 13776: pCAG-Cre:GFP

Gene/insert name:	Cre-GFP fusion protein
Alternative names:	Cre recombinase
Insert size (bp):	1784
Species of gene(s):	Bacteriophage P1
Fusion proteins or tags:	GFP
Terminal:	C terminal on insert
Vector backbone:	pCAGEN (Search Vector Database)
Type of vector:	Mammalian expression
Backbone size (bp):	4779
Cloning site 5':	EcoRI
Site destroyed during cloning:	No
Cloning site 3':	NotI
Site destroyed during cloning:	No
5' Sequencing primer:	pCAG-F (List of Sequencing Primers)
Bacteria resistance:	Ampicillin
High or low copy:	High Copy
Grow in standard E. coli @ 37C:	Yes
If you **did not originally clone** this gene, from whom and where did you receive the plasmid used to derive this plasmid:	The coding sequence of Cre was amplified by PCR using pxCANCre (Kanegae et al. NAR 23, 3816-3821 (1995)) obtained from Dr. Saito I (Univ. of Tokyo) as a template. GFP was from pEGFP-N1 (Clontech).
Sequence:	View sequence
Author's Map:	View map
Plasmid Provided In:	DH5a
Principal Investigator:	Connie Cepko
Terms and Licenses:	MTA

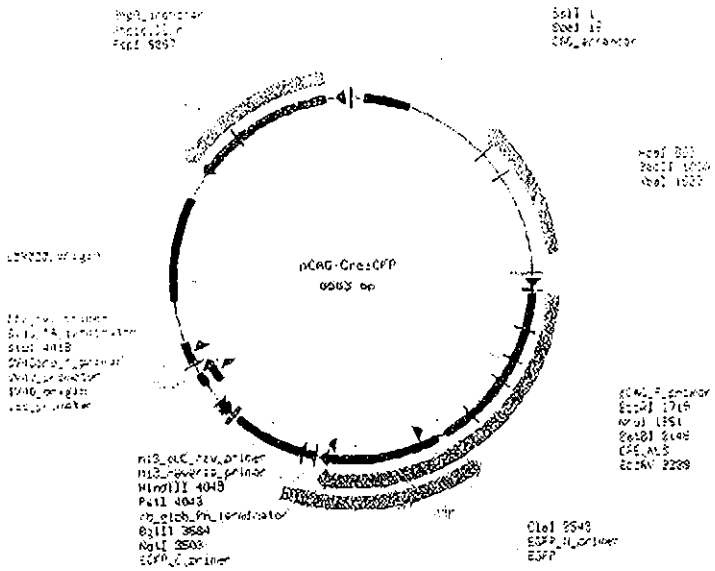
Comments: Kozak consensus sequence was added before the start ATG. GFP-tag was added at the C-terminus of Cre. Cre-GFP fusion protein is localized in the cell nucleus.

Addgene has sequenced a portion of this plasmid for verification. Click here for the sequencing result.

Click on map to enlarge

Plasmid Links
Author's map
Sequence
Reviews (0)
Related Plasmids
From this article
Connie Cepko Lab Plasmids

This is commonly requested with
pCAG-ERT2CreERT2
pCAG-Cre
pCALNL-DsRed
pCAG-CreERT2
pCAG-GFP



Selected features		
CAG_enhancer	84 - 371	→
ORF frame 1	1533 - 802	←
pCAG_F_primer	1678 - 1697	→
CRE_NLS	1749 - 2744	→
ORF frame 3	1737 - 3500	→
EGFP_N_primer	2847 - 2826	←
EGFP	2781 - 3497	→
ORF frame 3	3636 - 2649	←
EGFP_C_primer	3434 - 3455	→
rb_glob_PA_terminator	3519 - 4020	→
M13_reverse_primer	4060 - 4062	←
M13_pUC_rev_primer	4101 - 4079	←
lac_promoter	4144 - 4115	←
SV40_origin	4265 - 4362	→
SV40_promoter	4250 - 4429	→
SV40pro_F_primer	4347 - 4366	→
SV40_PA_terminator	4443 - 4574	→
EBV_rev_primer	4531 - 4550	←
pBR322_origin	5417 - 4798	←
ORF frame 1	6432 - 5572	←
Ampicillin	6432 - 5572	←
AmpR_promoter	6502 - 6474	←

Unique restriction sites	
SalI	1
SpeI	18
ApaI	863
SacII	1030
XbaI	1623
EcoRI	1719
NruI	1951
BstBI	2146
EcoRV	2286
ClaI	2543
NotI	3503
BglII	3584
PstI	4043
HindIII	4048
StuI	4418
FspI	5867

Article: Controlled expression of transgenes introduced by in vivo electroporation. Matsuda T et al. (Proc Natl Acad Sci U S A. 2007 Jan 5. ():. 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 13776" in your Materials and Methods section

addgene

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Plasmid ...

psPAX2
Plasmid 12260

pMD2.G
Plasmid 12259

Addgene Order 31497

Thank you for placing an order at Addgene. Please print a copy of the Order Receipt and/or Billing Invoice for your records.

Order Status:

- Your payment information is incomplete. Please submit payment information
- Waiting for MTA from your institution.

Want to make changes to this order? Click here.

Material Transfer Agreement

Your request requires a Material Transfer Agreement (MTA). Click here to see Frequently Asked Questions about Addgene's MTA process.

How to expedite the process: Addgene will work with the following person at your institution to process your MTA. Majority of the Addgene MTAs are approved within one to two business days. If your MTA is not approved after two business days, Addgene encourages you to contact the following person to follow up. If your institution requires you to route the MTA for approval, please find a copy of the MTA here.

Coordinator Name: Nancy McCreery
Coordinator Email: nancy.mccreery@uwo.ca
Coordinator Phone: 519-661-4254

Completed Forms:

File

Recipient Scientist Acknowledgment (3558 bytes)

Order ID: 31497

09/30/2009 11:54 EDT

Order Summary

ID	Plasmid Name	Subtotal
13776	pCAG-Cre:GFP	$65 00
Shipping and Handling		$45.00
Total:		$110.00

Shipping Address

Recipient:	Dr. Dagnino
Institution:	University of Western Ontario
Street:	Dental Science Stores Dock 15
Street:	Physiology and Pharmacology
City:	London
State/Province:	ON
Zip/Postal Code:	N6A 5C1
Country:	CANADA
Phone:	5196612111

Billing Address

Card Holder/Payer:	Dr. Dagnino
Institution:	University of Western Ontario
Street:	Support Services Building
Street:	Suite 6100
City:	London
State/Province:	ON
Zip/Postal Code:	N6A 3K7
Country:	CANADA
Phone:	5196612038

Payment Information

Payment Method: Pay Later

To: TAMES (University of Western Ontario, PI: Dagnino)

Vendor: Addgene Inc.
1 Kendall Square Ste B6302, Cambridge, MA 02139-1666
Internet: www.addgene.org E-mail: help@addgene.org Phone: (617) 225-9000

To contact us with questions about this order, please include the order ID in your e-mail.

Modification Form for Permit BIO-UWO-0008

Permit Holder: Lina Dagnino

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

Lylia Nini
David Judah
Ernest Ho
Timothy Irvine
Kerry-Ann Nakrieko

Additional Personnel
(Please list additional personnel here)

Randeep Singh
Amanda MacGillivary

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Approved Cells	Human (primary), rodent (primary), human (established) HeLa, HEK 293, Rodent (established) NIH-3T3 fibroblasts	HEK 293T cells MEL-5 melanoma cells
Approved Use of Human Source Material		
Approved GMO	Adenovirus	
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: Sep 24, 2007

Signature of Permit Holder: Lina Dagnino

BioSafety Officer(s): J Stanley Jan 30/09

Chair,Biohazards Subcommittee: E M Fidler

Modification Form for Permit BIO-UWO-0008

Permit Holder: Lina Dagnino

Approved Toxin(s)

Cholera toxin	

Other reagents requested for approval:
The following plasmids:

pMD2.G
psPAX2

Special considerations: Use of cholera toxin → Please follow the "Biosecurity Requirements for Facilities Using Biological Agents" attached. For more information, please see www.uwo.ca/human resources/biosafety.

* 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: Sep 24, 2007

Signature of Permit Holder: Lina Dagnino

BioSafety Officer(s): JStanley 9ab 30/09

Chair, Biohazards Subcommittee: G.M. Gold

HEK 293T: They are used to produce adenovirus, and also to conduct experiments in the laboratory that allow overexpression of proteins.

The MEL-5 cell line will be used to study mechanisms of transfer of melanin-containing granules into epidermal keratinocytes.
The plasmids will be used to produce vectors encoding shRNAs to knockdown protein expression in target cells.

Western

Biosecurity Requirements for Facilities Using Biological Agents

(1) Biological agents protected by a lock. For example, biological agents in a freezer, fridge, laboratories or other type of container must be locked after-hours/if no one present.

(2) The supervisor must ensure that each person has the qualifications and training to do the work without supervision.

(3) Visitors must be accompanied.

(4) The supervisor must keep a current inventory and a list of the location(s) where the biological agent(s) are stored and handled.

(5) Labelling to identify samples and the container in which they are stored.

(6) Notify the biosafety officer if a sample is lost, stolen, or otherwise misused.

(7) Notify Campus Community Police Services of suspicious behaviour.

There are two additional requirements for Facilities Using or Storing Biological Toxins:

(8) Do not keep on hand more than the amounts regulated by the United States Select Agents regulation: www.selectagents.gov/index.htm/

(9) For best practices, it is recommended to use or handle less than one human dose at any given time.

Summary of Approvals for Permit BIO-UWO-0008

Permit Holder: Lina Dagnino

Approved Personnel (Please stroke out any personnel to be removed

Ernest Ho

Timothy Irvine

Kerry-Ann Nakrieko

~~Agnieszka Pajak~~

~~Iordauka Ivanova~~

~~Wing Chang~~

Additional Personnel

David Judah

Lylia Nini

	Please stroke out any approved Biohazards* to be removed below	Write additional Biohazards for approval below.
Approved Microorganisms*	E. Coli DH5 alpha	
Approved Cells*	Human (primary), rodent (primary), human (established) HeLa, HEK 293, Rodent (established) NIH-3T3 fibroblasts	
Approved Use of Human Source Material*		
Approved GMO*	Adenovirus	
Approved use of Animals*		
Approved Toxin(s)*		Cholera Toxin

Date of last Biohazardous Agents Registry Form Sep 24, 2007

Signature of Permit Holder: Lina Dagnino

BioSafety Officer(s): JStanley / June 27/08

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

Summary of Approvals for Permit BIO-UWO-0008

Permit Holder: Lina Dagnino

Used as additive in culture medium for mouse primary keratinocytes

Date of last Biohazardous Agents Registry Form Sep 24, 2007

Signature of Permit Holder: Lina Dagnino

BioSafety Officer(s): [signature]

Chair, Biohazards Subcommittee G M Kidder 2 July 08

BIO - UWO-0008

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. LINA DAGNINO
SIGNATURE Lina Dagnino
DEPARTMENT Physiology & Pharmacology
ADDRESS 220 SDR1
PHONE NUMBER 84264
EMAIL ldagnino@uwo.ca

Location of experimental work to be carried out: Building(s) SDR1 Room(s) 220, 230, 231
*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):
Molecular Mechanisms of epididymal formation and regeneration

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. (Please see last page)

FUNDING AGENCY/AGENCIES CIHR

Names of all personnel working under Principal Investigators supervision in this location:
i) K. A. Nakrieko
ii) T. Irvine
iii) E. Ho
iv)
v)

* 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?
DH5α E. coli	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	2 L
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ 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)? Originally purchased

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	[redacted] (epithelial tumor)
Rodent	☑ Yes ☐ No	Epidermis
Non-human primate	☐ Yes ☒ No	
Other (specify)	None	

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	HeLa, HEK293	ATCC
Rodent	☑ Yes ☐ No	NIH-3T3 fibroblasts	Dr. Tini's lab
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

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

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_____
- 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: Adenovirus

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 _____

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 _____

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

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 Lisa Wagar

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: BIO-UWO-00008
20 June 2006
re-inspected 2007 ok

12.0 Approvals

UWO Biohazard Subcommittee

Signature E.M. Kidder Date 24 Sept. 07

Safety Officer for Institution where experiments will take place

Signature Jennifer Stanley Date Sept. 20/07

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*