

Modification Form for Permit BIO-RRI-0016

Permit Holder: Stephen Pasternak

Approved Personnel

(Please stroke out any personnel to be removed)

Badrinath Narayan
Adam Samosh
Angela Lorenzen
Robert Ta
Joshua Tam
Weihoa Tang
Claudia Seah

Additional Personnel

(Please list additional personnel here)

Approved
Microorganisms

Please stroke out any approved
Biohazards to be removed below

Write additional Biohazards for
approval below. Give the full name
- do not abbreviate.

E.coli DH5alpha

Approved Primary
and Established Cells

Human (established): HEK-293, SH-SY5Y,
Rodent (established): SN56. Rodent
(primary): mouse brain.

Approved Use of
Human Source
Material

CSF, brain

Approved Genetic
Modifications
(Plasmids/Vectors)

[plasmid]: based on pEGFP.

Tag RFP657
mTag BFP

Approved Use of
Animals

Approved Biological
Toxin(s)

* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.
** PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE 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: May 13, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): _____  Oct. 21, 2010

Chair, Biohazards Subcommittee: _____ Date: _____

These cDNAs encode fluorescent proteins which we attach to the Alzheimer's disease proteins we study. This allows us to directly see these proteins in liver cells under a fluorescent microscope. These proteins are mutants (minor variants) of fluorescent proteins we are already using.

Ron Noseworthy

From: Stephen Pasternak
Sent: Wednesday, October 20, 2010 1:34 PM
To: Ron Noseworthy
Subject: plasmids

Hi Ron,
I have not received forms from Jennifer Stanley.
Talk to you shortly
Steve

The plasmids encoding the proteins:

TagRFP657 (Red fluorescent protein)

mTagBFP (Blue fluorescent protein)

These cDNAs encode fluorescent proteins which we attach to the Alzheimer's disease proteins we study.

This allows us to directly see these protein in live cells under a fluorescent microscope.
These proteins are mutants (minor variants) of fluorescent proteins we are already using.

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
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 is 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 or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) 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), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR

Stephen M. Pasternak

SIGNATURE

DEPARTMENT

Clinical Neurological Sciences/ Physiology and Pharmacology/ Robarts

ADDRESS

RRI, 100 Perth Dr.

PHONE NUMBER

x25294 lab x24400, Clinic 646-6032

EMERGENCY PHONE NUMBER(S)

Pager 519-680-8312, Cell 519 673-8356

EMAIL

spasternak@robarts.ca

Location of experimental work to be carried out: Building(s) Robarts Room(s) 3218

*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 the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES: CIHR

GRANT TITLE(S): Characterizing the Endosomal/Lysosomal System in Alzheimer's disease.

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. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.

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

Claudia Seah

Weihoa Tang

Joshua Tam

Robert Ta

* 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 biological agents? YES NO
 (including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO
 If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____
 Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.
 Please describe any CFIA permit conditions:

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	PHAC or CFIA Containment Level
E.Coli DH5a	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	1 Liter		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 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 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:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	Mouse Brain	2008-060-05 pasternak
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> No		

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

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input checked="" type="radio"/> Yes <input type="radio"/> No	HEK-293, SH-SY5Y	Ferguson Lab
Rodent	<input checked="" type="radio"/> Yes <input type="radio"/> No	SN56	Rylett Lab
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input checked="" type="radio"/> 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 PHAC or CFIA containment level required 1 2 3

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

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid	Human CSF	<input type="radio"/> Yes <input checked="" type="radio"/> No <input type="radio"/> Unknown	NA	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)	Brain	Not Applicable	NA	Not Applicable

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 modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results
E. Coli DH5a	Plasmids based on pEGFP (See attached)		APP, Presenilin, LAMP1, compartment marker Proteins	used for growing up DNA only

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

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

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

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES HEK 293 NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
 Please attach a full description of the biological agent.

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

5.3 How will the biological agent 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, number: _____ NO PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used SD Rat, Transgenic Mouse model of Alzheimer's disease Mouse

6.3 AUS protocol # 2008-060-05 Pastenak

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? YES NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO
If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO
If NO, please forward the permit to the Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____
If no, please proceed to Section 12.0 NO

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

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

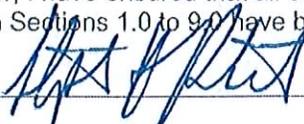
11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 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 (Western or equivalent)
- ◆ 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 _____


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

13.0 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. 1 2 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, permit # if on-campus BIO-RR1-0016
 NO, please certify
 NOT REQUIRED for Level 1 containment

14.0 Procedures to be Followed

14.1 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 (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 [Signature] Date: May 11 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:
Express the wound, and then flush the area with soap and water, and follow up with Staff health.

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: [Signature]
Date: 14 May 2010

Safety Officer for Institution where experiments will take place: SIGNATURE: [Signature]
Date: May 11, 2010

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: [Signature]
Date: May 14, 2010

Approval Number: BIO-RR1-0016 Expiry Date (3 years from Approval): May 13 2013

Special Conditions of Approval:

Characterizing the Role of the Endosomal/Lysosomal System in Alzheimer's Disease

A hallmark of Alzheimer's Disease (AD) is the deposition of amyloid plaques composed of 38-43 amino acid Amyloid-Beta Peptide ($A\beta$) in the brain. $A\beta$ is produced by the sequential proteolytic cleavage of the Amyloid Precursor Protein (APP), first by a β -secretase (BACE), and then by a γ -secretase complex (includes: Presenilin, Nicastrin, mAph1 and Pen2). Although many studies have implicated the endosomal/lysosomal system in the cleavage of APP to $A\beta$, currently there is no consensus as to where these events occur. Furthermore, the subcellular trafficking pathways of APP, which regulate where APP interacts with the secretase enzymes to produce $A\beta$, and the organelles responsible for $A\beta$ secretion have not been well characterized.

We have found that highly purified lysosomes from the rat liver and mouse brain are enriched in APP along with mature nicastrin, presenilin-1 (PS-1), mAph1 and Pen2, suggesting that the lysosome might be an important compartment for $A\beta$ generation. We have discovered three completely novel subcellular trafficking pathways, able to transport APP 1) directly from the cell surface to the lysosome, 2) from Golgi to the lysosome, and 3) from the lysosome to the cell surface. Furthermore, the direct transport of APP from the cell surface to the lysosome is unique in that it possesses a sorting system able to exclude APP mutants (Swedish and London) which cause familial Alzheimer's disease. **Therefore, we hypothesize that the endosomal/ lysosomal system is a major site of APP cleavage into $A\beta$ and that the mechanism of trafficking to and from the lysosome regulates $A\beta$ production and secretion.**

SPECIFIC AIMS: We propose to use live cell imaging techniques including confocal microscopy and Total Internal Reflection Microscopy (TIRFM) to address these specific aims:

1. To determine the mechanism underlying the rapid transport of APP directly from the cell surface to lysosomes.
2. To assess the effects of London and Swedish mutations on the intracellular trafficking of APP between the Golgi, lysosome and cell surface.
3. To identify the subcellular compartment required for the association and cleavage of APP by the γ -secretase complex.

We have prepared N-terminal epitope-tagged APP expression constructs (also containing C-terminal fluorescent protein tags) which allow us to label wild-type, Swedish and London variants of APP with fluorescent antibodies at the cell surface. We will then follow their internalization and trafficking in real time. Subcellular compartments will be identified using a panel of fluorescent protein-tagged markers for the early endosomal (rab5), late endosomal (rab9), lysosomal (LAMP1), Golgi (GalT) and endoplasmic reticulum (KDEL_mRDP). We will introduce genetic (i.e. Rac1, Arf6, Dynamin dominant negative) and pharmacological inhibitors of clathrin-mediated internalization, lipid raft internalization, and macropinocytosis to identify the mode(s) of wild type and mutant APP transport from the cell surface to the lysosome.

The trafficking of APP from internal compartments (i.e. the Golgi) will be studied using APP fused photoactivatable GFP (PAGFP). This allows us to 'turn on' green fluorescence of APP-PAGFP in the Golgi (identified using GalT-CFP) and follow its trafficking to LAMP1-mRFP labeled lysosomes. Transport of APP, $A\beta$ and lysosomal proteins to the cell surface will be studied in cells expressing fluorescent tagged APP, or loaded with fluorescent $A\beta$ induced to exocytosis by ionomycin.

The sites of APP/ γ -secretase interaction will be identified using Fluorescence Resonance Energy Transfer (FRET) after photobleaching, and the Proximity Ligation Assay. We will then use confocal microscopy to study the cleavage and clearance rate of fluorescent-tagged APP in specific subcellular compartments with and without the addition of specific γ -secretase inhibitor L685,485.

The proposal outlined here will elucidate 3 novel neuronal trafficking pathways and their relationship to APP processing and secretion. It will identify the site of APP interaction with and cleavage by the γ -secretase to produce $A\beta$. This work will add a new chapter to basic neuronal cell biology and provide insight into the basic mechanism of β -amyloid production. A better understanding of this critical process will aid in the design of therapeutic strategies for AD.

Plasmids used in this project are based on this plasmid backbone

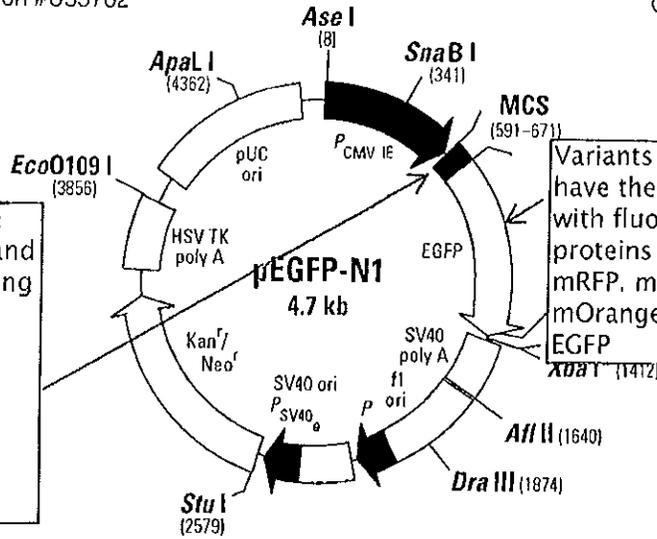
pEGFP-N1 Vector Information

GenBank Accession #U55762

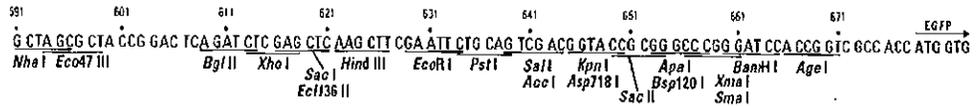
PT3027-5

Catalog #6085-1

cDNA's cloned into this site include the Amyloid Precursor Protein (full length and truncated forms, and Alzheimer's causing mutant forms), Presenilin 1 (and Alzheimer's causing mutant forms), Transported genes Rac1, Arf6, and compartment marker proteins such as rab2, rab3, rab5, rab9, LAMP1, GalT.



Variants of this plasmid have the GFP replaced with fluorescent proteins CFP, YFP, mRFP, mCherry and mOrange in place of EGFP



Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1 Vector. All restriction sites shown are unique. The *Not*I site follows the EGFP stop codon. The *Xba*I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ and make fresh DNA.

Description

pEGFP-N1 encodes a red-shifted variant of wild-type GFP (1-3) which has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 507 nm.) pEGFP-N1 encodes the GFPmut1 variant (4) which contains the double-amino-acid substitution of Phe-64 to Leu and Ser-65 to Thr. The coding sequence of the EGFP gene contains more than 190 silent base changes which correspond to human codon-usage preferences (5). Sequences flanking EGFP have been converted to a Kozak consensus translation initiation site (6) to further increase the translation efficiency in eukaryotic cells. The MCS in pEGFP-N1 is between the immediate early promoter of CMV (*P_{CMV IE}*) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neo'), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex virus thymidine kinase (HSV TK) gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette expresses kanamycin resistance in *E. coli*. The pEGFP-N1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.



United States/Canada
800.552.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3504.6880
Japan
+81.(0)77543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

(PR29972; published 03 October 2002)

Use

Fusions to the N terminus of EGFP retain the fluorescent properties of the native protein allowing the localization of the fusion protein *in vivo*. The target gene should be cloned into pEGFP-N1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. The inserted gene should include the initiating ATG codon. The recombinant EGFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (7). pEGFP-N1 can also be used simply to express EGFP in a cell line of interest (e.g., as a transfection marker).

Location of features

- Human cytomegalovirus (CMV) immediate early promoter: 1–589
Enhancer region: 59–465; TATA box: 554–560
Transcription start point: 583
C→G mutation to remove *Sac* I site: 569
- MCS: 591–671
- Enhanced green fluorescent protein (EGFP) gene
Kozak consensus translation initiation site: 672–682
Start codon (ATG): 679–681; Stop codon: 1396–1398
Insertion of Val at position 2: 682–684
GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 871–876
His-231 to Leu mutation (A→T): 1373
- SV40 early mRNA polyadenylation signal
Polyadenylation signals: 1552–1557 & 1581–1586; mRNA 3' ends: 1590 & 1602
- f1 single-strand DNA origin: 1649–2104 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene:
–35 region: 2166–2171; –10 region: 2189–2194
Transcription start point: 2201
- SV40 origin of replication: 2445–2580
- SV40 early promoter
Enhancer (72-bp tandem repeats): 2278–2349 & 2350–2421
21-bp repeats: 2425–2445, 2446–2466 & 2468–2488
Early promoter element: 2501–2507
Major transcription start points: 2497, 2535, 2541 & 2546
- Kanamycin/neomycin resistance gene
Neomycin phosphotransferase coding sequences: start codon (ATG): 2629–2631; stop codon: 3421–3423
G→A mutation to remove *Pst* I site: 2811
C→A (Arg to Ser) mutation to remove *Bss*H II site: 3157
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
Polyadenylation signals: 3659–3664 & 3672–3677
- pUC plasmid replication origin: 4008–4651

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 745–724
- EGFP-C Sequencing Primer (#6478-1): 1332–1353

Propagation in *E. coli*

- Suitable host strains: DH5a, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM101 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 µg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References:

1. Prasher, D. C., *et al.* (1992) *Gene* 111:229–233.
2. Chalfie, M., *et al.* (1994) *Science* 263:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* 341:277–280.
4. Cormack, B., *et al.* (1996) *Gene* 173:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* 6:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* 15:8125–8148.
7. Gorman, C. (1985). In *DNA cloning: A practical approach, vol. II*. Ed. D.M. Glover. (IRL Press, Oxford, U.K.) pp. 143–190.

Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

Notice to Purchaser

Use of BD Biosciences Clontech's Living Colors™ products containing DNA sequences coding for mutant *Aequorea victoria* green fluorescent protein (GFP) variants or proteins thereof requires a license from Amersham Biosciences under U.S. Patent Nos. 5,625,048; 5,777,079; 6,054,321 and other pending U.S. and foreign patent applications. In addition, certain BD Biosciences Clontech products are made under U.S. Patent No. 5,804,387 licensed from Stanford University.

Not-For-Profit research institutes or entities are granted an automatic license with the purchase of this product for use in non-commercial internal research purposes, the terms of which are disclosed in detail in the license that accompanies the shipment of this product. Such license specifically excludes the right to sell or otherwise transfer this product or its components to third parties.

For-Profit research institutes or entities must obtain a license from Amersham Biosciences. E-mail: gfp@amershambiosciences.com

Please contact BD Biosciences Clontech directly for any other assistance, including purchasing and technical support. All companies and institutions purchasing Living Colors™ products will be included in a quarterly report to Aurora Biosciences, as required by the BD Biosciences Clontech/Aurora Biosciences license agreement.

This product is intended to be used for research purposes only. It is not to be used for drug or diagnostic purposes nor is it intended for human use. BD Biosciences Clontech products may not be resold, modified for resale, or used to manufacture commercial products without written approval of BD Biosciences Clontech.

© 2002, Beclon, Dickinson and Company

Cell Biology

ATCC® Number:	CRL-2266™	Order this Item	Price:	\$272.00
Designations:	SH-SY5Y		Related Links ▶	
Depositors:	JL Biedler		NCBI Entrez Search	
<u>Biosafety Level:</u>	1		Cell Micrograph	
Shipped:	frozen		Make a Deposit	
Medium & Serum:	See Propagation		Frequently Asked Questions	
Growth Properties:	mixed, adherent and suspension		Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human)		Technical Support	
Morphology:	epithelial		Related Cell Culture Products	
				
Source:	Organ: brain Disease: neuroblastoma Derived from metastatic site: bone marrow			
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.			
Restrictions:	NOTE: SH-SY5Y was deposited at the ATCC by June L. Biedler, Memorial Sloan-Kettering Cancer Center. SH-SY5Y is distributed for academic research purposes only. Memorial Sloan-Kettering releases the line subject to the following: 1.) SH-SY5Y or its products must not be distributed to third parties. Commercial interests are the exclusive property of Memorial Sloan-Kettering Cancer Center. 2.) Any proposed commercial use of SH-SY5Y including any use by a for-profit entity must first be negotiated with Director, Office of Industrial Affairs, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY 10021; phone (212) 639-6181; FAX (212) 717-3439.			
Isolation:	Isolation date: 1970			
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)			
Antigen Expression:	Blood Type A; Rh+			

Amelogenin: X
CSFIPO: 11
D13S317: 11
D16S539: 8,13
DNA Profile (STR): D5S818: 12
D7S820: 7,10
TH01: 7,10
TPOX: 8,11
vWA: 14,18

Cytogenetic Analysis: modal number = 47; the cells possess a unique marker comprised of a chromosome 1 with a complex insertion of an additional copy of a 1q segment into the long arm, resulting in trisomy of 1q [[22554](#)]

Age: 4 years
Gender: female

Comments: SH-SY5Y cells have a reported saturation density greater than 1×10^6 cells/sq cm. They are reported to exhibit moderate levels of dopamine beta hydroxylase activity [PubMed ID: 29704].

Propagation: **ATCC complete growth medium:** The base medium for this cell line is a 1:1 mixture of ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003, and F12 Medium. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%
Temperature: 37.0°C

Subculturing: **Protocol:** These cells grow as a mixture of floating and adherent cells. The cells grow as clusters of neuroblastic cells with multiple, short, fine cell processes (neurites). Cells will aggregate, form clumps and float. Remove the medium with the floating cells, and recover the cells by centrifugation. Rinse the adherent cells with fresh 0.25% trypsin, 0.53 mM EDTA solution, add an additional 1 to 2 ml of trypsin solution, and let the culture sit at room temperature (or at 37C) until the cells detach. Add fresh medium, aspirate, combine with the floating cells recovered above and dispense into new flasks.
Subcultivation Ratio: A subcultivation ratio of 1:20 to 1:50 is recommended
Medium Renewal: Every 4 to 7 days

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

Doubling Time: 48 hrs

Related Products: recommended serum: [ATCC 30-2020](#)
parental cell line: [ATCC HTB-11](#)

References:

- 22554: Ross RA, et al. Coordinate morphological and biochemical interconversion of human neuroblastoma cells. J. Natl. Cancer Inst. 71: 741-749, 1983. PubMed: [6137586](#)
- 23032: Biedler JL, et al. Multiple neurotransmitter synthesis by human neuroblastoma cell lines and clones. Cancer Res. 38: 3751-3757, 1978. PubMed: [29704](#)

[Return to Top](#)

Cell Biology

ATCC® Number:	CRL-1573™ Order this Item	Price:	\$256.00
Designations:	293 [HEK-293]	Related Links ▶	
Depositors:	FL Graham	NCBI Entrez Search	
Biosafety Level:	2 [CELLS CONTAIN ADENOVIRUS]	Cell Micrograph	
Shipped:	frozen	Make a Deposit	
Medium & Serum:	See Propagation	Frequently Asked Questions	
Growth Properties:	adherent	Material Transfer Agreement	
Organism:	<i>Homo sapiens</i> (human) epithelial	Technical Support	
Morphology:	 PHOTO	Related Cell Culture Products	
Source:	Organ: embryonic kidney Cell Type: transformed with adenovirus 5 DNA		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		
Restrictions:	These cells are distributed for research purposes only. 293 cells, their products, or their derivatives may not be distributed to third parties.		
Applications:	efficacy testing [92587] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) virucide testing [92579]		
Receptors:	vitronectin, expressed		
Tumorigenic:	Yes		
DNA Profile (STR):	Amelogenin: X CSFIPO: 11,12 D13S317: 12,14 D16S539: 9,13 D5S818: 8,9 D7S820: 11,12 THO1: 7,9.3 TPOX: 11 vWA: 16,19		

Cytogenetic Analysis: This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %. The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired. There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.

Age: fetus

Comments: Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. [39768]
The line is excellent for titrating human adenoviruses. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. [23406]
The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2). [39768]

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: fetal bovine serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO₂), 5%
Temperature: 37.0°C
The cell line does not adhere to the substrate when left at room temperature for any length of time, therefore, live cultures may be received with the cells detached. The cells will re-attach to the flask over a period of several days in culture at 37C.

Protocol:

- Subculturing:
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. An inoculum of 2×10^3 to 6×10^3 viable cells/cm² is recommended.
 6. Incubate cultures at 37°C. 6. Subculture when cell concentration is between 6 and 7×10^4 cells/cm².

Subcultivation Ratio: 1:10 to 1:20 weekly.

Medium Renewal: Every 2 to 3 days

Preservation: **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO

Storage temperature: liquid nitrogen vapor phase

Recommended medium (without the additional supplements or serum described under ATCC Medium): ATCC 30-2003

derivative: ATCC CRL-10852

derivative: ATCC CRL-12006

derivative: ATCC CRL-12007

Related Products: derivative: ATCC CRL-12013

derivative: ATCC CRL-12479

derivative: ATCC CRL-2029

derivative: ATCC CRL-2368

purified DNA: ATCC CRL-1573D

21624: Xie QW, et al. Complementation analysis of mutants of nitric oxide synthase reveals that the active site requires two hemes. Proc. Natl. Acad. Sci. USA 93: 4891-4896, 1996.

PubMed: 8643499

21631: Da Costa LT, et al. Converting cancer genes into killer genes. Proc. Natl. Acad. Sci. USA 93: 4192-4196, 1996.

PubMed: 8633039

22282: Graham FL, et al. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36: 59-72, 1977. PubMed: 886304

22319: Graham FL, et al. Defective transforming capacity of adenovirus type 5 host-range mutants. Virology 86: 10-21, 1978. PubMed: 664220

22699: Harrison T, et al. Host-range mutants of adenovirus type 5 defective for growth in HeLa cells. *Virology* 77: 319-329, 1977. PubMed: [841862](#)

23406: Bodary SC, McLean JW. The integrin beta 1 subunit associates with the vitronectin receptor alpha v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. *J. Biol. Chem.* 265: 5938-5941, 1990. PubMed: [1690718](#)

27819: Goodrum FD, Ornelles DA. The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle. *J. Virol.* 71: 548-561, 1997. PubMed: [8985383](#)

28301: Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. *J. Virol.* 71: 42-49, 1997. PubMed: [8985321](#)

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](#)

32396: Kolanus W, et al. alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule. *Cell* 86: 233-242, 1996. PubMed: [8706128](#)

32490: Stauderman KA, et al. Characterization of human recombinant neuronal nicotinic acetylcholine receptor subunit combinations alpha 2 beta 4, alpha 3 beta 4 and alpha 4 beta 4 stably expressed in HEK293 cells. *J. Pharmacol. Exp. Ther.* 284: 777-789, 1998. PubMed: [9454827](#)

32514: Bartz SR, et al. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. *J. Virol.* 70: 2324-2331, 1996. PubMed: [8642659](#)

32726: Sandri-Goldin RM, Hibbard MK. The herpes simplex virus type 1 regulatory protein ICP27 coimmunoprecipitates with anti-sm antiserum, and the C terminus appears to be required for this interaction. *J. Virol.* 70: 108-118, 1996. PubMed: [8523514](#)

32829: Ansieau S, et al. Tumor necrosis factor receptor-associated factor (TRAF)-1, TRAF-2, and TRAF-3 interact in vivo with the CD30 cytoplasmic domain; TRAF-2 mediates CD30-induced nuclear factor kappa B activation. *Proc. Natl. Acad. Sci. USA* 93: 14053-14058, 1996. PubMed: [8943059](#)

32893: Zhang J, et al. Dynamain and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. *J. Biol. Chem.* 271: 18302-18305, 1996. PubMed: [8702465](#)

32914: Oppermann M, et al. Monoclonal antibodies reveal receptor specificity among G-protein-coupled receptor kinases. *Proc. Natl. Acad. Sci. USA* 93: 7649-7654, 1996. PubMed: [8755530](#)

32921: Xia Y, et al. Nitric oxide synthase generates

References:

- superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc. Natl. Acad. Sci. USA 93: 6770-6774, 1996. PubMed: [8692893](#)
- 32925: Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: [8650224](#)
- 32971: Uebele VN, et al. Functional differences in Kv1.5 currents expressed in mammalian cell lines are due to the presence of endogenous Kvbeta2.1 subunits. J. Biol. Chem. 271: 2406-2412, 1996. PubMed: [8576199](#)
- 33003: Abell A, et al. Deletions of portions of the extracellular loops of the lutropin/choriogonadotropin receptor decrease the binding affinity for ovine luteinizing hormone, but not human choriogonadotropin, by preventing the formation of mature cell surface receptor. J. Biol. Chem. 271: 4518-4527, 1996. PubMed: [8626807](#)
- 33010: Tiberi M, et al. Differential regulation of dopamine D1A receptor responsiveness by various G protein-coupled receptor kinases. J. Biol. Chem. 271: 3771-3778, 1996. PubMed: [8631993](#)
- 33022: Shahrestanifar M, et al. Studies on inhibition of mu and delta opioid receptor binding by dithiothreitol and N-ethylmaleimide. His223 is critical for mu opioid receptor binding and inactivation by N-ethylmaleimide. J. Biol. Chem. 271: 5505-5512, 1996. PubMed: [8621408](#)
- 33035: Boring L, et al. Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1alpha receptors. J. Biol. Chem. 271: 7551-7558, 1996. PubMed: [8631787](#)
- 33036: Noonberg SB, et al. Evidence of post-transcriptional regulation of U6 small nuclear RNA. J. Biol. Chem. 271: 10477-10481, 1996. PubMed: [8631843](#)
- 33050: Fox JC, Shanley JR. Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells. J. Biol. Chem. 271: 12578-12584, 1996. PubMed: [8647868](#)
- 33056: Lee MJ, et al. The inducible G protein-coupled receptor edg-1 signals via the Gi/mitogen-activated protein kinase pathway. J. Biol. Chem. 271: 11272-11279, 1996. PubMed: [8626678](#)
- 33123: Marchand P, et al. Cysteine mutations in the MAM domain result in monomeric meprin and alter stability and activity of the proteinase. J. Biol. Chem. 271: 24236-24241, 1996. PubMed: [8798668](#)
- 33137: Arai H, Charo IF. Differential regulation of G-protein-mediated signaling by chemokine receptors. J. Biol. Chem. 271: 21814-21819, 1996. PubMed: [8702980](#)
- 33138: Huang Q, et al. Substrate recognition by tissue factor-factor VIIa. J. Biol. Chem. 271: 21752-21757, 1996. PubMed:

8702971

33157: Monteclaro FS, Charo IF. The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1alpha receptor, confers chemokine selectivity. J. Biol. Chem. 271: 19084-19092, 1996. PubMed: 8702581

33158: Keith DE, et al. Morphine activates opioid receptors without causing their rapid internalization. J. Biol. Chem. 271: 19021-19024, 1996. PubMed: 8702570

39768: Louis N, et al. Cloning and sequencing of the cellular-viral junctions from the human adenovirus type 5 transformed 293 cell line. Virology 233: 423-429, 1997. PubMed: 9217065

61259: Shaw G, et al. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. FASEB J. 16: 869-871, 2002. PubMed: 11967234

92579: Standard Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash and Handrub Agents Using the Fingerpads of Adult Volunteers. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 1838-02.

92587: Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.

[Return to Top](#)