Modification Form for Permit BIO-RRI-0035

Permit Holder: Mike Strong

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

Wendy Strong

May Gohar

Wencheng Yang

Kathy Volkening

Danae Campos-Melo

Brian Keller

Cheryl Leystra-Lantz

Cristian Droppelmann

Additional Personnel
(Please list additional personnel here)

Jessica Kao

	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 (DH5 alpha, BL21, XL1 Blue, SCS 110) S. cerevisiae (AH09, Y187)	
Approved Primary and Established Cells	Human [established]: HEK-293T, IMR32, HCN1A. Rodent [established]: Neuro2A, PC12, EOC20, NSC34, BV2, LADMAC, L929/292.	Human - HeLa Mouse - NIH/3T3
Approved Use of Human Source Material	Human organs or tissues (unpreserved)	
Approved Genetic Modifications (Plasmids/Vectors)	SV 40 Large T antigen, E1A oncogene. [Plasmids]: pAS2-1, pBluescript SK9(-), pBridge, pcDNA3.1(+), pcDNA3.1/myc-HisA, pcDNA3.1/myc-HisB, pcDNA3.1/myc-HisC, pCMV-SPORT6, pCMX, pCRII-TOPO, see	
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: Aug 27, 2010

Date of Last Modification (if applicable): _____

BioSafety Officer(s): Ronald Nosaoff Nov.09.2010

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

HeLa cells to be used for transcient transfection studies as per the HEK293T and Neuro2A cell lines.

NIH/3T3 cells to be used for transient tranfection studies for GEF activity

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Product Description

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Cell Biology

ATCC® Number: CRL-1658™ Order this Item

Price: $256.00

Designations: NIH/3T3

Biosafety Level: 1

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Mus musculus* (mouse)

Morphology: fibroblast

Source:
Organ: embryo
Strain: NIH/Swiss
Cell Type: fibroblast fibroblast;

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.

Applications: transfection host (Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)

Virus Susceptibility: Murine leukemia virus

Age: embryo

Comments: The NIH/3T3 is highly sensitive to sarcoma virus focus formation and leukemia virus propagation and has proven to be very useful in DNA transfection studies (PubMed ID: 222457).
Tested and found negative for ectromelia virus (mousepox).

Propagation:
ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: bovine calf serum to a final concentration of 10%.
Atmosphere: air, 95%; carbon dioxide (CO2), 5%
Temperature: 37.0°C
Growth Conditions: The serum used is important in culturing this line. Calf serum is recommended and not fetal bovine serum. The calf serum initially employed and found to be satisfactory was from the Colorado Serum Co. Denver.

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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 which 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 37C 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 37C.

DO NOT ALLOW THE CELLS TO BECOME CONFLUENT! Subculture at least twice per week at 80% confluence or less.
Subcultivation Ratio: Inoculate 3 to 5 X 10(3) cells/cm2
Medium Renewal: Twice per week

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002

References:

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Cell Biology

ATCC® Number: CCL-2™ Order this Item

Price: $256.00

Designations: HeLa

Depositors: WF Scherer

Biosafety Level: 2 [Cells contain human papilloma virus]

Shipped: frozen

Medium & Serum: See Propagation

Growth Properties: adherent

Organism: *Homo sapiens* (human)

Morphology: epithelial

PHOTO

Source:
Organ: cervix
Disease: adenocarcinoma
Cell Type: epithelial

Cellular Products:
keratin
Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]

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.

Applications:
transfection host ([21491] Nucleofection technology from Lonza
Roche FuGENE® Transfection Reagents)
screening for Escherichia coli strains with invasive potential [21447] [21491]

Virus Susceptibility:
Human adenovirus 3
Encephalomyocarditis virus
Human poliovirus 1
Human poliovirus 2
Human poliovirus 3

DNA Profile (STR):
Amelogenin: X
CSF1PO: 9,10
D13S317: 12,13.3
D16S539: 9,10
D5S818: 11,12
D7S820: 8,12
THO1: 7
TPOX: 8,12
vWA: 16,18

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Cytogenetic Analysis: Modal number = 82; range = 70 to 164.
There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of MI, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.

Isoenzymes: G6PD, A

Age: 31 years adult

Gender: female

Ethnicity: Black

HeLa Markers: Y

Comments: The cells are positive for keratin by immunoperoxidase staining.
HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences.
P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.

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 (CO_2), 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 which 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 1:6 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: Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003
Also see 30-2020
derivative:ATCC CCL-2.1
derivative:ATCC CCL-2.2
derivative:ATCC CCL-2.3

References:

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BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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 Biohazards 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	**Dr Michael Strong**
DEPARTMENT	Molecular Brain Research Group/ Robarts Research Institute
ADDRESS	Room 3270 Robarts Research Institute
PHONE NUMBER	519-663-5777 x24452
EMERGENCY PHONE NUMBER(S)	Kathy Volkening 519-639-3802 or Cheryl Leystra-Lantz 519-452-1081
EMAIL	kmcdouga@uwo.ca lantz@robarts.ca

Location of experimental work to be carried out: Building(s) **Robarts Research Institute** Room(s) **3270**

*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 15.0, Approvals).

FUNDING AGENCY/AGENCIES: **see Appendix A**
GRANT TITLE(S): _____

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Kathryn Volkening	kmcdouga@uwo.ca	30-May-2007
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Zhong Ping He	zhe@robarts.ca	20-Jan-2009
Brian Keller	bakeller1@gmail.com	16-Sep-2009

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Constructs and plasmids and yeast2hybrid libraries (series of constructs within yeast): Constucts/plasmids are circular pieces of DNA that encode necessary components to allow for expression of a specific DNA/RNA or protein from it when expressed in cells (tissue culture, E coli or yeast, depending on the construct). These are non-replicable pieces of DNA when outside an organism and can only be expressed in a cell if certain pieces of the DNA match the host. None of our constructs/plasmids are viral in origin and none are infective, nor can they be used to create an infective construct/plasmid. All constructs that have been transformed to carrier E coli strains or yeast are stored as glycerol stocks (overnight transformed E coli culture in LB plus the appropriate selective antibiotic-ampicillin, kanamycin or tetracycline diluted with 50% glycerol) at -80 degrees C under lock and key. These glycerol stocks contain both the plasmid and the E coli strain into which is was transformed for ease of growth. The freezer in which these are stored is marked with the appropriate biohazardous materials symbol. Constructs and plasmids that are produced for short term storage and use (grown from the glycerol stocks above, and then the DNA is isolated from the E coli for subsequent use in procedures such as PCR or cloning) are stored as DNA in either water or Tris-EDTA pH 8.0 at -20 C or +4 C. These DNA are non-infective, non-pathogenic. Glycerol stocks are generally stored and never disposed of, however, in the event that they would be, they are thawed to room temperature, diluted 1:1 with bleach for 1 hour and disposed of down the fume hood drain. Any isolated DNA/constructs/plasmids are used in entirety in subsequent experiments, however, if required to dispose of them, these DNA are non-replicable outside a host and in such small quantities (generally 30ul or less) are either autoclaved or bleached prior to disposal. Use of E coli transformed with constructs and plasmids consists of handling with level 1 biosafety precautions. These E coli are used for the propogation of plasmid, long term storage of plasmid, or for production of GST fused proteins from the constructs. Plasmids/constructs themselves are used in subsequent experiments including PCR amplification of DNA pieces encoded within the contrucst/plasmids, restriction digests to free pieces of the DNA for subsequent subcloning, transfection of constructs/plasmids for expression in tissue culture cells to examine gene expression. Yeast2hybrid constructs/libraries are stored as glycerol stocks (as above) and used to determine the interaction of proteins within the yeast2 hybrid system. Cloning and yeast2hybrid systems commonly require plating of transformed E coli or yeast onto either LB/agar or minimal synthetic dropout medium/agar plates. These cultures are disposed of in the biosafety waste stream and autoclaved to destroy them prior to disposal using Robarts Research's autoclaving service.

E coli and yeast strains: These are common hosts used to propagate, store and express DNA constructs/plasmids. The strains that are used in this lab are non-pathogenic. E coli are stored as glycerol stocks (described above-but without any plasmid or construct transformed into them). Yeast are also stored as glycerol stocks (again, non-transformed). They are handled with standard biosafety level I precautions. For disposal (which is extremely rare) cultures are bleached as above and disposed of down the drain. Any plates that have been used to grow E coli or yeast are autoclaved prior to disposal.

All tissue culture cells: Mammalian tissue culture cells are the backbone of our in vitro assay systems for examining the expression of genes (from plasmids above), protein interactions, and the effects of mutations on a living system without requiring animal use. All tissue culture cells are handled with biosafety level II precautions in an approved laminar flow hood. Cultures are maintained in dedicated incubators marked with biosafety labeling. Stored stocks of culture cells are in liquid nitrogen, in freezing medium (containing 50% DMSO, serum and DMEM culture medium). Short term stock storage (ie, during the freezing process is at -80C). All cultures are used for either control or experimental group studies for gene/protein expression. Cells are commonly transfected with the constructs/plasmid described above. All extra or unused cells (other than those in storage) are bleached for 1 hour prior to disposal down the drain. The work area is spot cleaned as necessary with bleach and the finally disinfected after use with 70% ethanol or isopropanol. Tissue culture cells that are transfected with constructs above are rarely disposed of, they are used in subsequent experiments in which they are lysed in standard lysis buffers and then used in PAGE analysis for expression of proteins. Any remaining samples after lysis (at which point the cells are destroyed) are either autoclaved or bleached prior to disposal.

Human brain and spinal cord tissues (unfixed, frozen):
Unfixed brain and spinal cord from human ALS patients and controls are stored under lock and key at -80C in an appropriately labeled freezer. These tissues are stored either in closed capped containers labeled with the tissue or are double bagged and labeled. Only authorized persons (those working directly with these tissues in their experiments and having undergone training) are permitted access to these tissues. Tissues are handled by only persons trained in biohazard level II techniques and they are handled in a regular fume hood. All tissues are maintained and not disposed of after use, only tissues needed for an experiment are removed from storage for immediate use. All materials to come in contact with the tissues are bleached or autoclaved prior to disposal including all tubes, tips, dishes and dissection materials. As stated, all samples of these types are used in subsequent experiments (IP, PAGE, protein interaction, DNA analysis, RNA analysis etc) and any remaining unused tissues are stored as above.

Please include a one page research summary or teaching protocol.

Dr. Strong's lab researches the molecular and biochemical basis of amyotrophic lateral sclerosis (ALS). ALS is a deadly disease hallmarked by the formation of neurofilament aggregates within neurons, followed by the loss of motor neurons, and leading to pronounced, progressive paralysis and death commonly within 2-5 years from the date of diagnosis. Recently there have been several genes determined to play a role in the development of ALS, of which several mutations seem to contribute. However, the functional significance of these mutations is unclear. It is of great interest to this lab that many of these proteins are also involved in the processes to make, stabilize, transport and degrade messenger RNA, the intermediate between genetic coding and protein production, within a cell. It is becoming increasingly clear that many neurodegenerative diseases appear to be diseases of RNA processing, and the roles that these proteins play in this process is the main focus of the research in this lab. A secondary focus of the lab is the etiology of Tau containing tangles in neurodegenerative disease. Tau protein can be found to be abnormally deposited in neurodegenerative disease states, which can also contribute to neuron death.

The etiology of Tau containing tangles: This study aims at determining how this protein forms tangles within the brain. Immunohistochemical studies are aimed at determining the distribution of these tangles throughout the brain. Biochemical properties of the mechanism of tangle formation are also being investigated through the use of mutations introduced into the gene and determining their effect on tau polymerization both in vitro and in tissue culture cells. Protein polymerization is governed by the phosphorylation status of a protein. By altering the pattern of phosphorylation on tau constructs that we create and then express in culture cells or as GST fused proteins, we can determine which phosphorylation event is pathological and leads to tangle formation.

RNA stability in ALS: This set of projects aims to determine the role of RNA binding proteins in the mechanism of aggregate formation, as well as altered metabolism, excitotoxicity and cell death in ALS. It is becoming increasingly clear that the proteins that bear mutations in ALS are also involved in the RNA metabolism pathways, and alterations in their function(s) within this path can have dire consequences on the health of cells. Through our previous research we have determined that low molecular weight neurofilament (NFL; a protein required for motor neuron survival and function) mRNA is significantly altered in ALS. Similar alterations when produced in mice leads to the formation of aggregates containing related neurofilament proteins. The presence of an intracellular aggregate then predisposes the neuron to excitotoxicity which can lead to cell death. To date many of the proteins known to be altered in expression or bear mutations in ALS have been found to be RNA binding proteins which play a direct role in determining the stability of NFL or other mRNA species. In addition, microRNA (miRNA) can also profoundly affect the stability of mRNA, commonly targeting the mRNA for degradation prior to protein production. In rare cases, miRNA can stabilize a mRNA, preventing degradation while holding that mRNA in a quiescent state without protein production. In another level of complexity, a family of small non-coding RNA called microRNA (miRNA) have been described to directly influence the stability of mRNA, most commonly through allowing for destruction of the mRNA prior to its translation to protein, but rarely to stabilize RNA in a translationally quiescent manner until it is required by the cell. Ongoing research focuses on the role that mutations in the RNA binding proteins play in altering the stability of NFL mRNA, as well as determining the expression patterns of miRNA in ALS, and how alterations in levels of miRNA may contribute to the NFL mRNA decreases seen in ALS.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? **x** YES O NO
(non-pathogenic and pathogenic biological agents 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? O YES **x** 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 DH5α *E. coli* BL21	O Yes **x** No	O Yes **x** No	O Yes **x** No	500ml	Invitrogen	**x** 1 O 2 O 2+ O 3
E. coli XL1 Blue SCS 110	O Yes **x** No	O Yes **x** No	O Yes **x** No	100ml	Stratagene	**x** 1 O 2 O 2+ O 3
S.cerevisiae strains AH09 and Y187	O Yes **x** No	O Yes **x** No	O Yes **x** No	200ml	Clontech	**x** 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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? **x** 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:

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

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	**x** Yes O No	HEK-293T, IMR32, HCN1A	ATCC
Rodent	**x** Yes O No	Neuro2A, PC12, EOC20, NSC34, BV2, LADMAC, L929/292	Cashman *et al* 1992, ATCC Blasi *et al* 1990
Non-human primate	O Yes **x** No		
Other (specify)	O Yes **x** 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 **x** 1 **x** 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? **x** YES O 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		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)	LHSC	O Yes **x** Unknown		O 1 **x** 2 O 2+ O 3
Human Organs or Tissues (preserved)	LHSC	Not Applicable		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? **x** YES O NO If no, please proceed to Section 5.0

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

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
	see Appendix B			.

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

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

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

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ **x** NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ **x** NO
- SV 40 Large T antigen **HEK 293T cells** **x**.YES O NO
- E1A oncogene **HEK 292T cells** **x** YES O NO
- Known oncogenes O YES, please specify _____ **x** NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ **x** NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES **x** 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

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

6.2 Name of animal species to be used_____

6.3 AUS protocol # _____

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

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

_____

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES **x** No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

8.0 Biological Toxins

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

8.2 If YES, please name the toxin(s)_____
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_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES **x** NO If no, please proceed to Section 10.0

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

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES **x** 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? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O 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? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ **x** NO
If no, please proceed to Section 12.0

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

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ **x** 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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____ 26 July 2010

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 **x** 2 O 2+ O 3

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

x YES, permit # if on-campus **BIO-RRI-0035**
O NO, please certify
O 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 _____ Date: 26 Aug 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 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 biological agents listed, such as a needlestick injury:

No animal or needles used in lab.

_____

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: Susan F. Koval
Date: August 27, 2010

2) Safety Officer for the University of Western Ontario
SIGNATURE: J Stanley
Date: Aug 27, 2010

3) Safety Officer for Institution where experiments will take place (~~if not~~ UWO):
SIGNATURE: _____
Date: August 05, 2010

Approval Number: BIO-RRI-0035 Expiry Date (3 years from Approval): Aug 20 2013

Special Conditions of Approval:

Appendix A:

1) FUNDING AGENCY: Canadian Institutes of Health Research
GRANT TITLE: MicroRNA (miRNA) modulation of NFL mRNA degradation and translational silencing in ALS, 2010 – 2013.

2) FUNDING AGENCY: ALS Society of Canada Senior Investigator Bridge Funding
GRANT TITLE: MicroRNA (miRNA) modulation of NFL mRNA degradation and translational silencing in ALS: a novel window to the understanding of RNA stability in ALS, 2009.

3) FUNDING AGENCY: Canadian Institutes of Health Research
GRANT TITLE: The role of TDP-43 in regulating NFL mRNA metabolism, 2008-2011.

Plasmid	Notes	Supplier
pAS2-1	bait protein for Y2H	Clontech #K1604-B
pBluescript SK(-)	cloning with blue/white screening, T7, T3	Agilent
pBridge	yeast, two MCS's	Clontech #630404
pcDNA3.1(+)	mammalian expression, T7	Invitrogen #V790-20
pcDNA3.1/myc-HisA	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pcDNA3.1/myc-HisB	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pcDNA3.1/myc-HisC	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pCMV-SPORT6	generation of cDNA libraries	Invitrogen #12209-011
pCMX	constituative mammalian expression	unknown
pCRII-TOPO	cloning, Sp6, T7	Invitrogen K4650-01
pCR-XL-TOPO	cloning of long PCR products	Invitrogen K4750-10
pECFP-C1	fusion protein at C-terminus of CFP @475nm	Clontech #6076-1
pDsRed1-N1	fusion protein at N-terminus of RFP @582nm	Clontech 6921-1
pEGFP-C1	fusion protein at C-terminus of GFP @507nm	Clontech #6084-1
pEGFP-N1	fusion protein at N-terminus of GFP @507nm	Clontech #6085-1
pEYFP-C1	fusion protein at C-terminus of YFP @527nm	Clontech #6005-1
pEYFP-N1	fusion protein at N-terminus of YFP @527nm	Clontech #6006-1
pGAD-424	yeast shuttle vector	Clontech #K1605-1
pGAD-T7	yeast shuttle vector + HA tag, T7	Clontech #K1612-1
pGAD-T7-Rec	cDNA library construction for Y2H	Clontech #630490
pGBKT7-53	positive control for Y2H	Clontec #630445
pGBKT7-DNA-BD	GAL4 DNA-BD+Myc+Fusion protein	Clontech #630443
pGBKT7-LAM	negative control for bkgd inY2H	Clontech #630445
pGEM-4Z	cloning with blue/white screening, T7, SP6	Promega # P2161
pGEM-7Zf(+)	cloning, T7, Sp6	Promega #P2251
pGEM-7Zf(-)	cloning, T7, Sp6	Promega #P2371
pGEM-T Easy	cloning with blue/white screening, T7, SP6	Promega #E1360
pGEX-2T	GST-fusion/thrombin cleavage	GE Healthcare 28-9546-53
pGEX-4T2	GST-fusion/thrombin cleavage	GE Healthcare 28-9545-50
pGEX5X-3	GST-fusion/factor Xa cleavage	GE Healthcare 28-9545-55
pGL3-Control	luciferase reporter vector	Promega E1741
pIRES-EGFP	fusion+IRES +YFP	Clontech #6064-1
pMIR GLO	Dual-Luciferase miRNA Target Expression	Promega #E1330
pOTB7	cloning, CAM^R, T7, SP6	unknown
pRFP-N1	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRFP-N2	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRFP-N3	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRK172	cloning, T7	Dr DP Hanger
pRSVi	cloning	Dr R. Liem
pSuper	endogenous production of siRNA	Oligoengine VEC-PBS-0001/0002

Plasmid	Notes	Supplier
pT7T3D	cloning, T3, T7	Image
pTET-Off Advanced	to develop stableTet-Off cell lines	Clontech #631126
pTRE-Tight	response plasmid expressing gene	Clontech #631126
pTRE Tight Luc	expresses luciferase	Clontech #631126
pTRIamp19	T3, T7 and Sp6 promotors	Ambion 7424

Section 1.0 Microorganisms

Section 1.2 Biological Agents

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 18265017
Product name Subcloning Efficiency ™ DH5alpha™ Competent Cells

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
5250 MAINWAY DRIVE
BURLINGTON, ONT
CANADA L7L 6A4
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

For research use only

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components
The product contains no substances which at their given concentration, are considered to be hazardous to health. We recommend handling all chemicals with caution.

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

3. HAZARDS IDENTIFICATION

Form
Liquid

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	May be harmful if swallowed.

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water. If symptoms persist, call a physician.
Eye contact	Rinse thoroughly with plenty of water, also under the eyelids. If symptoms persist, call a physician.
Ingestion	Never give anything by mouth to an unconscious person. If symptoms persist, call a physician.
Inhalation	Move to fresh air. If symptoms persist, call a physician.
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory Protection In case of insufficient ventilation wear suitable respiratory equipment

Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability Stable.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Principle Routes of Exposure/ Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available

Ingestion	May be harmful if swallowed.

Specific effects **(Long Term Effects)**

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects	No information available.
Mobility	No information available.
Biodegradation	Inherently biodegradable.
Bioaccumulation	Does not bioaccumulate.

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name	Not classified as dangerous in the meaning of transport regulations
Hazard Class	No information available
Subsidiary Class	No information available
Packing group	No information available
UN-No	No information available

15. REGULATORY INFORMATION

International Inventories

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

For research use only

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since the Company cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESSED OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTICULAR PURPOSE.

End of Safety Data Sheet

invitrogen™

Material Safety Data Sheet

Revision Date: 24-Mar-2008

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND THE COMPANY/UNDERTAKING

Product code 500149
Product name BL21 (DE3) One Shot

Company/Undertaking Identification

INVITROGEN CORPORATON
5791 VAN ALLEN WAY
PO BOX 6482
CARLSBAD, CA 92008
760-603-7200

INVITROGEN CORPORATION
2270 INDUSTRIAL STREET
BURLINGTON, ONT
CANADA L7P 1A1
800-263-6236

GIBCO PRODUCTS
INVITROGEN CORPORATION
3175 STALEY ROAD P.O. BOX 68
GRAND ISLAND, NY 14072
716-774-6700

24 hour Emergency Response (Transport): 866-536-0631
301-431-8585
Outside of the U.S. ++1-301-431-8585

2. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous/Non-hazardous Components

Chemical Name	CAS-No	Weight %
Glycerol	56-81-5	10-30

3. HAZARDS IDENTIFICATION

Emergency Overview
The product contains no substances which at their given concentration, are considered to be hazardous to health

Form
Liquid

3. HAZARDS IDENTIFICATION

Principle Routes of Exposure/ Potential Health effects

Eyes	No information available
Skin	No information available
Inhalation	No information available
Ingestion	No information available

Specific effects

Carcinogenic effects	No information available
Mutagenic effects	No information available
Reproductive toxicity	No information available
Sensitization	No information available

Target Organ Effects No information available

HMIS

Health	0
Flammability	0
Reactivity	0

4. FIRST AID MEASURES

Skin contact	Wash off immediately with plenty of water
Eye contact	Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes
Ingestion	Never give anything by mouth to an unconscious person
Inhalation	Move to fresh air
Notes to physician	Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media	Dry chemical
Special protective equipment for firefighters	Wear self-contained breathing apparatus and protective suit

6. ACCIDENTAL RELEASE MEASURES

Personal precautions	Use personal protective equipment
Methods for cleaning up	Soak up with inert absorbent material.

7. HANDLING AND STORAGE

Handling	No special handling advice required
Storage	Keep in properly labelled containers

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Occupational exposure controls

Exposure limits

Chemical Name	OSHA PEL (TWA)	OSHA PEL (Ceiling)	ACGIH OEL (TWA)	ACGIH OEL (STEL)
Glycerol	15 mg/m³ total dust 5 mg/m³ respirable fraction	-	10 mg/m³	-

Engineering measures Ensure adequate ventilation, especially in confined areas

Personal protective equipment

Respiratory protection In case of insufficient ventilation wear suitable respiratory equipment
Hand protection Protective gloves
Eye protection Safety glasses with side-shields
Skin and body protection Lightweight protective clothing.
Hygiene measures Handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls Prevent product from entering drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

General Information

Form Liquid

Important Health Safety and Environmental Information

Boiling point/range	**°C** No data available	**°F** No data available
Melting point/range	**°C** No data available	**°F** No data available
Flash point	**°C** No data available	**°F** No data available
Autoignition temperature	**°C** No data available	**°F** No data available
Oxidizing properties	No information available	
Water solubility	No data available	

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.
Materials to avoid No information available
Hazardous decomposition products No information available
Polymerization Hazardous polymerisation does not occur.

11. TOXICOLOGICAL INFORMATION

Acute toxicity

Chemical Name	LD50 (oral,rat/mouse)	LD50 (dermal,rat/rabbit)	LC50 (Inhalation,rat/mouse)
Glycerol	12600 mg/kg (Rat)	10 g/kg (Rabbit)	570 mg/m³ (Rat)

Principle Routes of Exposure/ Potential Health effects

Eyes No information available
Skin No information available
Inhalation No information available
Ingestion No information available

Specific effects

Carcinogenic effects No information available
Mutagenic effects No information available
Reproductive toxicity No information available

Sensitization No information available

Target Organ Effects No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity effects No information available.
Mobility No information available.
Biodegradation No information available.
Bioaccumulation No information available

13. DISPOSAL CONSIDERATIONS

Dispose of in accordance with local regulations

14. TRANSPORT INFORMATION

IATA

Proper shipping name Not classified as dangerous in the meaning of transport regulations
Hazard Class No information available
Subsidiary Class No information available
Packing group No information available
UN-No No information available

15. REGULATORY INFORMATION

International Inventories

Chemical Name	TSCA	PICCS	ENCS	DSL	NDSL	AICS
Glycerol	Listed	Listed	Listed	Listed	-	Listed

U.S. Federal Regulations

SARA 313
This product is not regulated by SARA.

Clean Air Act, Section 112 Hazardous Air Pollutants (HAPs) (see 40 CFR 61)
This product does not contains HAPs.

U.S. State Regulations

Chemical Name	Massachusetts - RTK	New Jersey - RTK	Pennsylvania - RTK	Illinois - RTK	Rhode Island - RTK
Glycerol	Listed	-	Listed	-	Listed

California Proposition 65
This product does not contain chemicals listed under Proposition 65

WHMIS hazard class:
Non-controlled

This product has been classified according to the hazard criteria of the CPR and the MSDS contains all of the information required by the CPR

16. OTHER INFORMATION

This material is sold for research and development purposes only. It is not for any human or animal therapeutic or clinical diagnostic use. It is not intended for food, drug, household, agricultural, or cosmetic use. An individual technically qualified to handle potentially hazardous chemicals must supervise the use of this material.

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may be present unknown hazards and should be used with caution. Since Invitrogen Corporation cannot control the actual methods, volumes, or conditions of use, the Company shall not be held liable for any damages or losses resulting from the handling or from contact with the product as described herein. THE INFORMATION IN THIS MSDS DOES NOT CONSTITUTE A WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR ANY PARTCULAR PURPOSE.

End of Safety Data Sheet

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

Stratagene XL1-Blue Competent Cells, Catalog #200249

1 . Product and company identification

Product name	:	**Stratagene XL1-Blue Competent Cells, Catalog #200249**
Part No.	:	pUC18 Control Plasmid DNA 200231-42 1.42 M 2-Mercaptoethanol 210200-43 XL1-Blue Competent Cells 200236-41
Manufacturer / Supplier	:	Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Emergency telephone number	:	1-800-894-1304
Use of the substance/preparation	:	Chemical Kit
Validation date	:	01/09/2009

2 . Hazards identification

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		XL1-Blue Competent Cells	Liquid.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
OSHA/HCS status	:	pUC18 Control Plasmid DNA	While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
		1.42 M 2-Mercaptoethanol	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
		XL1-Blue Competent Cells	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
Emergency overview-Signal Word	:	WARNING !	
Emergency overview-Label Statement	:	pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
		1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
		XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

2 . Hazards identification

	pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed. Irritating to eyes and skin. May cause sensitization by skin contact. Do not breathe vapor or mist. Do not ingest. Do not get on skin or clothing. Avoid contact with eyes. Wash thoroughly after handling.
	XL1-Blue Competent Cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage, based on animal data. Wash thoroughly after handling.
	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Routes of entry :	pUC18 Control Plasmid DNA	Eye contact. Ingestion.
	1.42 M 2-Mercaptoethanol	Dermal contact. Eye contact. Inhalation. Ingestion.
	XL1-Blue Competent Cells	Eye contact. Inhalation. Ingestion.
Potential acute health effects		
Eyes :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to eyes.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Skin :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
	XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion :	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
	1.42 M 2-Mercaptoethanol	Toxic if swallowed.
	XL1-Blue Competent Cells	Toxic if swallowed.
Medical conditions aggravated by over-exposure :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation.
	XL1-Blue Competent Cells	Repeated or prolonged exposure to the substance can produce target organs damage.
Over-exposure signs/symptoms :	pUC18 Control Plasmid DNA	Not applicable.
	1.42 M 2-Mercaptoethanol	Not applicable.
	XL1-Blue Competent Cells	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
1.42 M 2-Mercaptoethanol		
2-Mercaptoethanol	60-24-2	10
XL1-Blue Competent Cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

Eye contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	:	pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.

4. First aid measures

Ingestion	:	pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		XL1-Blue Competent Cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Notes to physician	:	No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.	

5. Fire-fighting measures

Flammability of the product	:	pUC18 Control Plasmid DNA	Non-flammable.
		1.42 M 2-Mercaptoethanol	Non-flammable.
		XL1-Blue Competent Cells	Non-flammable.
Products of combustion	:	pUC18 Control Plasmid DNA	No specific data.
		1.42 M 2-Mercaptoethanol	Decomposition products may include the following materials: carbon oxides sulfur oxides
		XL1-Blue Competent Cells	Decomposition products may include the following materials: carbon oxides sulfur oxides halogenated compounds metal oxide/oxides

Extinguishing media

Suitable	:	pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
		1.42 M 2-Mercaptoethanol	Use an extinguishing agent suitable for the surrounding fire.
		XL1-Blue Competent Cells	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

Personal precautions	:	pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		1.42 M 2-Mercaptoethanol	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		XL1-Blue Competent Cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
Environmental precautions	:	pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		1.42 M 2-Mercaptoethanol	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		XL1-Blue Competent Cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up			
Small spill	:	pUC18 Control Plasmid DNA	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		1.42 M 2-Mercaptoethanol	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		XL1-Blue Competent Cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling	:	pUC18 Control Plasmid DNA	Wash thoroughly after handling.
		1.42 M 2-Mercaptoethanol	Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
		XL1-Blue Competent Cells	Do not ingest. Wash thoroughly after handling.

7 . Handling and storage

Storage : Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
1.42 M 2-Mercaptoethanol	
2-Mercaptoethanol	**AIHA WEEL (United States, 1/2008).** TWA: 0.2 ppm 8 hour(s).
XL1-Blue Competent Cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust
Manganese dichloride	**ACGIH TLV (United States, 1/2008).** TWA: 0.2 mg/m³, (as Mn) 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** CEIL: 5 mg/m³, (as Mn) **NIOSH REL (United States, 12/2001).** TWA: 1 mg/m³, (as Mn) 10 hour(s). STEL: 3 mg/m³, (as Mn) 15 minute(s). **OSHA PEL (United States, 11/2006).** CEIL: 5 mg/m³, (as Mn)
Sucrose	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction **NIOSH REL (United States, 12/2001).** TWA: 10 mg/m³ 10 hour(s). Form: Total TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction **OSHA PEL (United States, 11/2006).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
Dimethyl sulfoxide	**AIHA WEEL (United States, 1/2008).** TWA: 250 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

8 . Exposure controls/personal protection

Skin	:	Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.
Respiratory	:	Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.
Hands	:	Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.
Other protection	:	Not available.
Hygiene measures	:	Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		XL1-Blue Competent Cells	Liquid.
Flash point	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		XL1-Blue Competent Cells	Not applicable.
Color	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.
pH	:	pUC18 Control Plasmid DNA	Neutral.
		1.42 M 2-Mercaptoethanol	Neutral.
		XL1-Blue Competent Cells	Neutral.
Boiling/condensation point	:	pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
		1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
		XL1-Blue Competent Cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F)
Melting/freezing point	:	pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
		1.42 M 2-Mercaptoethanol	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
		XL1-Blue Competent Cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 3.02°C (37.4°F)

9 . Physical and chemical properties

Relative density	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Only known value: 1.1 (Water = 1) (2-Mercaptoethanol).
		XL1-Blue Competent Cells	Weighted average: 1.29 (Water = 1)
Vapor pressure	:	pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C)
		XL1-Blue Competent Cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.11 kPa (15.83 mm Hg) (at 20°C)
Vapor density	:	pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1)
		XL1-Blue Competent Cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.98 (Air = 1)
Evaporation rate	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	0.026 (Dimethyl sulfoxide) compared with Butyl acetate.

10 . Stability and reactivity

Stability and reactivity	:	The product is stable.
Incompatibility with various substances	:	Highly reactive or incompatible with the following materials: oxidizing materials and organic materials. Reactive or incompatible with the following materials: acids.
Hazardous decomposition products	:	pUC18 Control Plasmid DNA: Under normal conditions of storage and use, hazardous decomposition products should not be produced. 1.42 M 2-Mercaptoethanol: Under normal conditions of storage and use, hazardous decomposition products should not be produced. XL1-Blue Competent Cells: Under normal conditions of storage and use, hazardous decomposition products should not be produced.
Conditions of reactivity - Flammability	:	Flammable in the presence of the following materials or conditions: open flames, sparks and static discharge.

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Dimethyl sulfoxide	LD50 Dermal	Rat	40 gm/kg	-
	LD50 Oral	Rat	14500 mg/kg	-
Sucrose	LD50 Oral	Rat	29700 mg/kg	-
Manganese dichloride	LD50 Oral	Rat	250 mg/kg	-
Glycerol	LD50 Dermal	Rabbit	>10 gm/kg	-
	LD50 Oral	Rat	12600 mg/kg	-
Potassium chloride	LD50 Oral	Rat	2600 mg/kg	-

Eyes	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to eyes.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.

11 . Toxicological information

Skin	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Inhalation	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
		XL1-Blue Competent Cells	No known significant effects or critical hazards.
Ingestion	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
		XL1-Blue Competent Cells	Toxic if swallowed.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
XL1-Blue Competent Cells						
Sucrose	A4	-	-	-	-	-

Potential chronic health effects

Chronic effects	:	Contains material that may cause target organ damage, based on animal data.
Carcinogenicity	:	No known significant effects or critical hazards.
Mutagenicity	:	No known significant effects or critical hazards.
Teratogenicity	:	No known significant effects or critical hazards.
Developmental effects	:	No known significant effects or critical hazards.
Fertility effects	:	No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation	:	No specific data.
Ingestion	:	No specific data.
Skin	:	No specific data.
Eyes	:	No specific data.

Target organs	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Other adverse effects	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		XL1-Blue Competent Cells	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

12 . Ecological information

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Dimethyl sulfoxide	-	Acute LC50 35 to 37 ml/L Fresh water	Fish	96 hours
	-	Acute LC50 34000000 ug/L Fresh water	Fish	96 hours
Manganese dichloride	-	Acute EC50 4700 ug/L Fresh water	Daphnia	48 hours
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Potassium chloride	-	Acute EC50 83000 ug/L Fresh water	Daphnia	48 hours
	-	Acute LC50 337 mg/L Fresh water	Daphnia	48 hours
	-	Acute LC50 435000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification	: pUC18 Control Plasmid DNA	Not regulated.
	1.42 M 2-Mercaptoethanol	Toxic material Irritating material Sensitizing material
	XL1-Blue Competent Cells	Toxic material Target organ effects

15 . Regulatory information

	pUC18 Control Plasmid DNA	Not available.
	1.42 M 2-Mercaptoethanol	Not available.
	XL1-Blue Competent Cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
U.S. Federal regulations :	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
	1.42 M 2-Mercaptoethanol	**United States inventory (TSCA 8b):** All components are listed or exempted.
	XL1-Blue Competent Cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
	pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
	1.42 M 2-Mercaptoethanol	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: 2-Mercaptoethanol **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard
	XL1-Blue Competent Cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose; Dimethyl sulfoxide **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard; Dimethyl sulfoxide: Immediate (acute) health hazard, Delayed (chronic) health hazard
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 307**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 307**: No products were found.
	pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 311**: Edetic acid
	1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 311**: No products were found.
	XL1-Blue Competent Cells	**Clean Water Act (CWA) 311**: No products were found.

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pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
XL1-Blue Competent Cells	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.

SARA 313

	Product name	CAS number	Concentration
Form R - Reporting requirements :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification :	**XL1-Blue Competent Cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations : pUC18 Control Plasmid DNA

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: None of the components are listed.
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the

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components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

1.42 M 2-Mercaptoethanol

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: 2-Mercaptoethanol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: 2-Mercaptoethanol
Rhode Island Hazardous Substances: None of the components are listed.

XL1-Blue Competent Cells

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol;Sucrose
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: The following components are listed: Manganese dichloride
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the

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components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride;Sucrose
Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

Label requirements	: pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
	1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
	XL1-Blue Competent Cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

Date of issue : 01/09/2009
Version : 1

Notice to reader

DISCLAIMER: This Material Safety Data Sheet is offered without charge to the clients of Agilent Technologies. Data is the most current available to Agilent Technologies at the time of preparation and is issued as a matter of information only, no warranty as to its accuracy or completeness is expressed or implied.

☑ **Indicates information that has changed from previously issued version.**

Conforms to ANSI Z400.5-2004 Standard (United States).

Material Safety Data Sheet

Agilent Technologies

Stratagene SCS110 Competent Cells, Catalog #200247

1 . Product and company identification

Product name	:	**Stratagene SCS110 Competent Cells, Catalog #200247**
Part No.	:	pUC18 Control Plasmid DNA 200231-42 1.42 M 2-Mercaptoethanol 210200-43 SCS110 competent cells 200247-41
Manufacturer / Supplier	:	Agilent Technologies, Inc. 1834 State Highway 71 West Cedar Creek, TX 78612
Emergency telephone number	:	1-800-894-1304
Use of the substance/preparation	:	Chemical Kit
Validation date	:	01/09/2009

2 . Hazards identification

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		SCS110 competent cells	Liquid.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Not available.
OSHA/HCS status	:	pUC18 Control Plasmid DNA	While this material is not considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200), this MSDS contains valuable information critical to the safe handling and proper use of the product. This MSDS should be retained and available for employees and other users of this product.
		1.42 M 2-Mercaptoethanol	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
		SCS110 competent cells	This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).
Emergency overview-Signal Word	:	WARNING !	
Emergency overview-Label Statement	:	pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
		1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
		SCS110 competent cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.
		pUC18 Control Plasmid DNA	No known significant effects or critical hazards. Avoid prolonged contact with eyes, skin and clothing.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed. Irritating to eyes and skin. May cause sensitization by skin contact. Do not breathe vapor or mist. Do not ingest. Do not get on skin or clothing. Avoid contact with eyes. Wash thoroughly after handling.
		SCS110 competent cells	Toxic if swallowed. Avoid exposure - obtain special instructions before use. Do not breathe vapor or mist. Do not ingest. Avoid contact with eyes, skin and clothing. Contains material that may cause target organ damage,

2 . Hazards identification

			based on animal data. Wash thoroughly after handling.
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Routes of entry	:	pUC18 Control Plasmid DNA	Eye contact. Ingestion.
		1.42 M 2-Mercaptoethanol	Dermal contact. Eye contact. Inhalation. Ingestion.
		SCS110 competent cells	Eye contact. Inhalation. Ingestion.
Potential acute health effects			
Eyes	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to eyes.
		SCS110 competent cells	No known significant effects or critical hazards.
Skin	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
		SCS110 competent cells	No known significant effects or critical hazards.
Inhalation	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
		SCS110 competent cells	No known significant effects or critical hazards.
Ingestion	:	pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
		1.42 M 2-Mercaptoethanol	Toxic if swallowed.
		SCS110 competent cells	Toxic if swallowed.
Medical conditions aggravated by over-exposure	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Repeated skin exposure can produce local skin destruction or dermatitis. Repeated or prolonged contact with spray or mist may produce chronic eye irritation and severe skin irritation.
		SCS110 competent cells	Repeated or prolonged exposure to the substance can produce target organs damage.
Over-exposure signs/symptoms	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		SCS110 competent cells	Not applicable.

See toxicological information (section 11)

3 . Composition/information on ingredients

Name	CAS number	%
1.42 M 2-Mercaptoethanol		
2-Mercaptoethanol	60-24-2	10
SCS110 competent cells		
Glycerol	56-81-5	5 - 10
Manganese dichloride	7773-01-5	5 - 10
Sucrose	57-50-1	5 - 10
Dimethyl sulfoxide	67-68-5	5 - 10
Potassium chloride	7447-40-7	1 - 5

3 . Composition/information on ingredients

There are no ingredients or additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

4 . First aid measures

Eye contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
		SCS110 competent cells	In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention if adverse health effects persist or are severe.
Skin contact	:	pUC18 Control Plasmid DNA	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
		SCS110 competent cells	In case of contact, immediately flush skin with plenty of water. Remove contaminated clothing and shoes. Wash clothing before reuse. Clean shoes thoroughly before reuse. Get medical attention if adverse health effects persist or are severe.
Inhalation	:	pUC18 Control Plasmid DNA	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
		SCS110 competent cells	If inhaled, remove to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Get medical attention if adverse health effects persist or are severe.
Ingestion	:	pUC18 Control Plasmid DNA	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		1.42 M 2-Mercaptoethanol	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
		SCS110 competent cells	Do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Get medical attention if adverse health effects persist or are severe.
Protection of first-aiders	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		SCS110 competent cells	Not applicable.

4 . First aid measures

Notes to physician : No specific treatment. Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

5 . Fire-fighting measures

Flammability of the product	:	pUC18 Control Plasmid DNA	Non-flammable.
		1.42 M 2-Mercaptoethanol	Non-flammable.
		SCS110 competent cells	Non-flammable.
Products of combustion	:	pUC18 Control Plasmid DNA	No specific data.
		1.42 M 2-Mercaptoethanol	Decomposition products may include the following materials: carbon oxides sulfur oxides
		SCS110 competent cells	Decomposition products may include the following materials: carbon oxides sulfur oxides halogenated compounds metal oxide/oxides

Extinguishing media

Suitable	:	pUC18 Control Plasmid DNA	Use an extinguishing agent suitable for the surrounding fire.
		1.42 M 2-Mercaptoethanol	Use an extinguishing agent suitable for the surrounding fire.
		SCS110 competent cells	Use an extinguishing agent suitable for the surrounding fire.
Not suitable	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		SCS110 competent cells	Not applicable.
Special protective equipment for fire-fighters	:	Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.	
Special remarks on fire hazards	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Not available.
Special remarks on explosion hazards	:	Not available.	

6 . Accidental release measures

Personal precautions	:	pUC18 Control Plasmid DNA	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		1.42 M 2-Mercaptoethanol	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).
		SCS110 competent cells	No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Do not touch or walk through spilled material. Avoid breathing vapor or mist. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment (see section 8).

6 . Accidental release measures

Environmental precautions	:	pUC18 Control Plasmid DNA	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		1.42 M 2-Mercaptoethanol	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
		SCS110 competent cells	Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).
Methods for cleaning up			
Small spill	:	pUC18 Control Plasmid DNA	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		1.42 M 2-Mercaptoethanol	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.
		SCS110 competent cells	Stop leak if without risk. Move containers from spill area. Dilute with water and mop up if water-soluble or absorb with an inert dry material and place in an appropriate waste disposal container. Dispose of via a licensed waste disposal contractor.

7 . Handling and storage

Handling	:	pUC18 Control Plasmid DNA	Wash thoroughly after handling.
		1.42 M 2-Mercaptoethanol	Do not ingest. Avoid contact with eyes, skin and clothing. Wash thoroughly after handling.
		SCS110 competent cells	Do not ingest. Wash thoroughly after handling.
Storage	:	Store in accordance with local regulations. Store in original container protected from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see section 10) and food and drink. Keep container tightly closed and sealed until ready for use. Containers that have been opened must be carefully resealed and kept upright to prevent leakage. Do not store in unlabeled containers. Use appropriate containment to avoid environmental contamination.	

8 . Exposure controls/personal protection

Product name	Exposure limits
United States	
1.42 M 2-Mercaptoethanol	
2-Mercaptoethanol	**AIHA WEEL (United States, 1/2008).** TWA: 0.2 ppm 8 hour(s).
SCS110 competent cells	
Glycerol	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). Form: Mist **OSHA PEL (United States, 11/2006).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 15 mg/m³ 8 hour(s). Form: Total dust **OSHA PEL 1989 (United States, 3/1989).** TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction TWA: 10 mg/m³ 8 hour(s). Form: Total dust

8 . Exposure controls/personal protection

Manganese dichloride	**ACGIH TLV (United States, 1/2008).** TWA: 0.2 mg/m³, (as Mn) 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** CEIL: 5 mg/m³, (as Mn) **NIOSH REL (United States, 12/2001).** TWA: 1 mg/m³, (as Mn) 10 hour(s). STEL: 3 mg/m³, (as Mn) 15 minute(s). **OSHA PEL (United States, 11/2006).** CEIL: 5 mg/m³, (as Mn)
Sucrose	**ACGIH TLV (United States, 1/2008).** TWA: 10 mg/m³ 8 hour(s). **OSHA PEL 1989 (United States, 3/1989).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction **NIOSH REL (United States, 12/2001).** TWA: 10 mg/m³ 10 hour(s). Form: Total TWA: 5 mg/m³ 10 hour(s). Form: Respirable fraction **OSHA PEL (United States, 11/2006).** TWA: 15 mg/m³ 8 hour(s). Form: Total dust TWA: 5 mg/m³ 8 hour(s). Form: Respirable fraction
Dimethyl sulfoxide	**AIHA WEEL (United States, 1/2008).** TWA: 250 ppm 8 hour(s).

Consult local authorities for acceptable exposure limits.

Engineering measures : If user operations generate dust, fumes, gas, vapor or mist, use process enclosures, local exhaust ventilation or other engineering controls to keep worker exposure to airborne contaminants below any recommended or statutory limits.

Personal protection

Eyes : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts.

Skin : Chemical resistant protective gloves and clothing are recommended. The choice of protective gloves or clothing must be based on chemical resistance and other use requirements. Generally, BUNA-N offers acceptable chemical resistance. Individuals who are acutely and specifically sensitive to this chemical may require additional protective clothing.

Respiratory : Use a properly fitted, air-purifying or air-fed respirator complying with an approved standard if a risk assessment indicates this is necessary. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Hands : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary.

Other protection : Not available.

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

9 . Physical and chemical properties

Physical state	:	pUC18 Control Plasmid DNA	Liquid.
		1.42 M 2-Mercaptoethanol	Liquid.
		SCS110 competent cells	Liquid.
Flash point	:	pUC18 Control Plasmid DNA	Not applicable.
		1.42 M 2-Mercaptoethanol	Not applicable.
		SCS110 competent cells	Not applicable.
Color	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Not available.
Odor	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Not available.
pH	:	pUC18 Control Plasmid DNA	Neutral.
		1.42 M 2-Mercaptoethanol	Neutral.
		SCS110 competent cells	Neutral.
Boiling/condensation point	:	pUC18 Control Plasmid DNA	Lowest known value: 100°C (212°F) (Water).
		1.42 M 2-Mercaptoethanol	Lowest known value: 100°C (212°F) (Water). Weighted average: 105.7°C (222.3°F)
		SCS110 competent cells	Lowest known value: 100°C (212°F) (Water). Weighted average: 122.01°C (251.6°F)
Melting/freezing point	:	pUC18 Control Plasmid DNA	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
		1.42 M 2-Mercaptoethanol	May start to solidify at the following temperature: 0°C (32°F) This is based on data for the following ingredient: Water.
		SCS110 competent cells	May start to solidify at the following temperature: 19.8°C (67.6°F) This is based on data for the following ingredient: Glycerol. Weighted average: 3.02°C (37.4°F)
Relative density	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Only known value: 1.1 (Water = 1) (2-Mercaptoethanol).
		SCS110 competent cells	Weighted average: 1.29 (Water = 1)
Vapor pressure	:	pUC18 Control Plasmid DNA	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.08 kPa (15.6 mm Hg) (at 20°C)
		SCS110 competent cells	Highest known value: 2.3 kPa (17.5 mm Hg) (at 20°C) (Water). Weighted average: 2.11 kPa (15.83 mm Hg) (at 20°C)
Vapor density	:	pUC18 Control Plasmid DNA	Highest known value: 0.62 (Air = 1) (Water).
		1.42 M 2-Mercaptoethanol	Highest known value: 2.7 (Air = 1) (2-Mercaptoethanol). Weighted average: 0.83 (Air = 1)
		SCS110 competent cells	Highest known value: 3.1 (Air = 1) (Glycerol). Weighted average: 0.98 (Air = 1)
Evaporation rate	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	0.026 (Dimethyl sulfoxide) compared with Butyl acetate.

10 . Stability and reactivity

Stability and reactivity : The product is stable.

Incompatibility with various substances : Highly reactive or incompatible with the following materials: oxidizing materials and organic materials.
Reactive or incompatible with the following materials: acids.

Hazardous decomposition products :

pUC18 Control Plasmid DNA	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
1.42 M 2-Mercaptoethanol	Under normal conditions of storage and use, hazardous decomposition products should not be produced.
SCS110 competent cells	Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Conditions of reactivity - Flammability : Flammable in the presence of the following materials or conditions: open flames, sparks and static discharge.

11 . Toxicological information

Acute toxicity

Product/ingredient name	Result	Species	Dose	Exposure
Dimethyl sulfoxide	LD50 Dermal	Rat	40 gm/kg	-
	LD50 Oral	Rat	14500 mg/kg	-
Sucrose	LD50 Oral	Rat	29700 mg/kg	-
Manganese dichloride	LD50 Oral	Rat	250 mg/kg	-
Glycerol	LD50 Dermal	Rabbit	>10 gm/kg	-
	LD50 Oral	Rat	12600 mg/kg	-
Potassium chloride	LD50 Oral	Rat	2600 mg/kg	-

Eyes :

pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
1.42 M 2-Mercaptoethanol	Irritating to eyes.
SCS110 competent cells	No known significant effects or critical hazards.

Skin :

pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
1.42 M 2-Mercaptoethanol	Irritating to skin. May cause sensitization by skin contact.
SCS110 competent cells	No known significant effects or critical hazards.

Inhalation :

pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
1.42 M 2-Mercaptoethanol	No known significant effects or critical hazards.
SCS110 competent cells	No known significant effects or critical hazards.

Ingestion :

pUC18 Control Plasmid DNA	No known significant effects or critical hazards.
1.42 M 2-Mercaptoethanol	Toxic if swallowed.
SCS110 competent cells	Toxic if swallowed.

Classification

Product/ingredient name	ACGIH	IARC	EPA	NIOSH	NTP	OSHA
SCS110 competent cells						
Sucrose	A4	-	-	-	-	-

Potential chronic health effects

Chronic effects : Contains material that may cause target organ damage, based on animal data.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Over-exposure signs/symptoms

Inhalation : No specific data.

Ingestion : No specific data.

11 . Toxicological information

Skin	:	No specific data.	
Eyes	:	No specific data.	
Target organs	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
Other adverse effects	:	pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Not available.

12 . Ecological information

Environmental effects : No known significant effects or critical hazards.

Aquatic ecotoxicity

Product/ingredient name	Test	Result	Species	Exposure
Dimethyl sulfoxide	-	Acute LC50 35 to 37 ml/L Fresh water	Fish	96 hours
	-	Acute LC50 34000000 ug/L Fresh water	Fish	96 hours
Manganese dichloride	-	Acute EC50 4700 ug/L Fresh water	Daphnia	48 hours
Glycerol	-	Acute LC50 54 to 57 ml/L Fresh water	Fish	96 hours
Potassium chloride	-	Acute EC50 83000 ug/L Fresh water	Daphnia	48 hours
	-	Acute LC50 337 mg/L Fresh water	Daphnia	48 hours
	-	Acute LC50 435000 ug/L Fresh water	Fish	96 hours

Other adverse effects : No known significant effects or critical hazards.

13 . Disposal considerations

Waste disposal : The generation of waste should be avoided or minimized wherever possible. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Avoid dispersal of spilled material and runoff and contact with soil, waterways, drains and sewers.

Disposal should be in accordance with applicable regional, national and local laws and regulations. Local regulations may be more stringent than regional or national requirements.

The information presented below only applies to the material as supplied. The identification based on characteristic(s) or listing may not apply if the material has been used or otherwise contaminated. It is the responsibility of the waste generator to determine the toxicity and physical properties of the material generated to determine the proper waste identification and disposal methods in compliance with applicable regulations.

Refer to Section 7: HANDLING AND STORAGE and Section 8: EXPOSURE CONTROLS/PERSONAL PROTECTION for additional handling information and protection of employees.

14 . Transport information

Regulatory information

DOT / IMDG / IATA : Not regulated.

15 . Regulatory information

HCS Classification	:	pUC18 Control Plasmid DNA	Not regulated.
		1.42 M 2-Mercaptoethanol	Toxic material Irritating material Sensitizing material
		SCS110 competent cells	Toxic material Target organ effects
		pUC18 Control Plasmid DNA	Not available.
		1.42 M 2-Mercaptoethanol	Not available.
		SCS110 competent cells	Contains material which may cause damage to the following organs: blood, kidneys, gastrointestinal tract, upper respiratory tract, skin, central nervous system (CNS), eye, lens or cornea.
U.S. Federal regulations	:	pUC18 Control Plasmid DNA	**United States inventory (TSCA 8b):** All components are listed or exempted.
		1.42 M 2-Mercaptoethanol	**United States inventory (TSCA 8b):** All components are listed or exempted.
		SCS110 competent cells	**United States inventory (TSCA 8b):** All components are listed or exempted.
		pUC18 Control Plasmid DNA	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: No products were found. **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: No products were found.
		1.42 M 2-Mercaptoethanol	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: 2-Mercaptoethanol **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: 2-Mercaptoethanol: Fire hazard, Immediate (acute) health hazard, Delayed (chronic) health hazard
		SCS110 competent cells	**SARA 302/304/311/312 extremely hazardous substances**: No products were found. **SARA 302/304 emergency planning and notification**: No products were found. **SARA 302/304/311/312 hazardous chemicals**: Potassium chloride; Glycerol; Manganese dichloride; Sucrose; Dimethyl sulfoxide **SARA 311/312 MSDS distribution - chemical inventory - hazard identification**: Potassium chloride: Immediate (acute) health hazard, Delayed (chronic) health hazard; Glycerol: Immediate (acute) health hazard, Delayed (chronic) health hazard; Manganese dichloride: Delayed (chronic) health hazard; Sucrose: Delayed (chronic) health hazard; Dimethyl sulfoxide: Immediate (acute) health hazard, Delayed (chronic) health hazard

15 . Regulatory information

pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 307**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 307**: No products were found.
SCS110 competent cells	**Clean Water Act (CWA) 307**: No products were found.
pUC18 Control Plasmid DNA	**Clean Water Act (CWA) 311**: Edetic acid
1.42 M 2-Mercaptoethanol	**Clean Water Act (CWA) 311**: No products were found.
SCS110 competent cells	**Clean Water Act (CWA) 311**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
SCS110 competent cells	**Clean Air Act (CAA) 112 accidental release prevention**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
SCS110 competent cells	**Clean Air Act (CAA) 112 regulated flammable substances**: No products were found.
pUC18 Control Plasmid DNA	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
1.42 M 2-Mercaptoethanol	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.
SCS110 competent cells	**Clean Air Act (CAA) 112 regulated toxic substances**: No products were found.

SARA 313

	Product name	CAS number	Concentration
Form R - Reporting requirements :	**SCS110 competent cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1
Supplier notification :	**SCS110 competent cells**		
	Manganese dichloride	7773-01-5	5 - 10
	Hexaamminecobalt trichloride	10534-89-1	0.1 - 1

SARA 313 notifications must not be detached from the MSDS and any copying and redistribution of the MSDS shall include copying and redistribution of the notice attached to copies of the MSDS subsequently redistributed.

State regulations :	pUC18 Control Plasmid DNA	**Connecticut Carcinogen Reporting**: None of the components are listed. **Connecticut Hazardous Material Survey**: None of the components are listed. **Florida substances**: None of the components are listed. **Illinois Chemical Safety Act**: None of the components are listed. **Illinois Toxic Substances Disclosure to Employee Act**: None of the components are listed. **Louisiana Reporting**: None of the components are listed. **Louisiana Spill**: None of the components are listed. **Massachusetts Spill**: None of the components are listed. **Massachusetts Substances**: None of the components are listed. **Michigan Critical Material**: None of the components are listed. **Minnesota Hazardous Substances**: None of the components are listed. **New Jersey Hazardous Substances**: None of the components are listed. **New Jersey Spill**: None of the components are listed.

15 . Regulatory information

New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: None of the components are listed.
Rhode Island Hazardous Substances: None of the components are listed.

1.42 M 2-Mercaptoethanol

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: 2-Mercaptoethanol
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: None of the components are listed.
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: 2-Mercaptoethanol
Rhode Island Hazardous Substances: None of the components are listed.

SCS110 competent cells

Connecticut Carcinogen Reporting: None of the components are listed.
Connecticut Hazardous Material Survey: None of the components are listed.
Florida substances: None of the components are listed.
Illinois Chemical Safety Act: None of the components are listed.
Illinois Toxic Substances Disclosure to Employee Act: None of the components are listed.
Louisiana Reporting: None of the components are listed.
Louisiana Spill: None of the components are listed.
Massachusetts Spill: None of the components are listed.
Massachusetts Substances: The following components are listed: Glycerol;Sucrose
Michigan Critical Material: None of the components are listed.
Minnesota Hazardous Substances: None of the components are listed.
New Jersey Hazardous Substances: The following

15 . Regulatory information

components are listed: Manganese dichloride
New Jersey Spill: None of the components are listed.
New Jersey Toxic Catastrophe Prevention Act: None of the components are listed.
New York Acutely Hazardous Substances: None of the components are listed.
New York Toxic Chemical Release Reporting: None of the components are listed.
Pennsylvania RTK Hazardous Substances: The following components are listed: Glycerol; Manganese dichloride;Sucrose
Rhode Island Hazardous Substances: None of the components are listed.

State regulations - California Prop. 65 : No products were found.

16 . Other information

Label requirements	: pUC18 Control Plasmid DNA	NOT EXPECTED TO PRODUCE SIGNIFICANT ADVERSE HEALTH EFFECTS WHEN THE RECOMMENDED INSTRUCTIONS FOR USE ARE FOLLOWED.
	1.42 M 2-Mercaptoethanol	HARMFUL IF SWALLOWED. CAUSES EYE AND SKIN IRRITATION. MAY CAUSE ALLERGIC SKIN REACTION.
	SCS110 competent cells	HARMFUL IF SWALLOWED. CONTAINS MATERIAL THAT MAY CAUSE TARGET ORGAN DAMAGE, BASED ON ANIMAL DATA.

Date of issue : 01/09/2009
Version : 1

Notice to reader

DISCLAIMER: This Material Safety Data Sheet is offered without charge to the clients of Agilent Technologies. Data is the most current available to Agilent Technologies at the time of preparation and is issued as a matter of information only, no warranty as to its accuracy or completeness is expressed or implied.

☑ **Indicates information that has changed from previously issued version.**

IV. Yeast Strains

For additional information on the growth and maintenance of yeast, see the Yeast Protocols Handbook (PT3024-1). We also recommend Guthrie & Fink's *Guide to Yeast Genetics and Molecular Biology* (1991) and Heslot & Gaillardin's *Molecular Biology and Genetic Engineering of Yeasts* (1992).

A. Genotypes

TABLE I. MATCHMAKER YEAST STRAIN GENOTYPES

Strain	Genotype[a]	References
AH109[b]	*MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4Δ, gal80Δ, LYS2 : : $GAL1_{UAS}$-$GAL1_{TATA}$-HIS3, $GAL2_{UAS}$-$GAL2_{TATA}$-ADE2, URA3 : : $MEL1_{UAS}$-$MEL1_{TATA}$-lacZ, MEL1*	James *et al.*, 1996; Our unpublished observations
Y187 Clontech # 630457	*MATα, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4Δ, met⁻, gal80Δ, URA3 : : $GAL1_{UAS}$-$GAL1_{TATA}$-lacZ, MEL1*	Harper *et al.*, 1993

[a] The *GAL1*, *GAL2*, and *MEL1* upstream activating sequences (UASs) are responsive to the GAL4 transcriptional activator. The *trp1*, *his3*, *gal4*, and *gal80* mutations are all deletions; *leu2-3, 112* is a double mutation.

[b] AH109 is a derivative of strain PJ69-2A and includes the *ADE2* and *HIS3* nutritional markers and an endogenous *MEL1* gene (James *et al.*, 1996). The *lacZ* reporter gene was introduced into PJ69-2A to create strain AH109.

B. Phenotypes

It is important to verify the phenotypes of the AH109 and Y187 strains (Table II).

1. To recover strains from frozen stock, scrape a small amount of cells from the surface with a sterile loop or wooden stick and streak them onto YPDA plates.
2. Incubate plates at 30°C for 3–5 days until colonies appear. Propagate additional cultures only from isolated colonies on this working stock plate.

   Notes:

   - AH109 (and transformants derived from this strain) should be maintained on adenine-supplemented YPD (i.e., YPDA) for optimal viability of the strain and to prevent selection of spontaneous *ade1* or *ade5* mutations (Guthrie & Fink, 1991).
   - If you cannot recover the strain by scraping the frozen stock, the cells may have settled to the bottom of the tube before the stock was frozen. If this happens, thaw the frozen culture on ice and vortex it before restreaking.
   - Although nonlibrary stock cultures may be thawed and refrozen several times without significantly decreasing the viability, we recommend that you divide the once-thawed stock into aliquots before you refreeze it. This will keep the viability higher and will reduce the risk of bacterial contamination.

3. Test for the nutritional requirements shown in Table II.
   a. Using a sterile loop or toothpick, streak 3–4 colonies from the working stock onto separate, appropriate SD selection plates.
   b. Incubate plates at 30°C for 4–6 days. Yeast grows slower on SD selection medium than on YPDA.
   c. Compare your results with those shown in Table II. Proceed only if AH109 and Y187 have the expected phenotypes.
4. Use well-isolated colonies from the verified working stock plate to inoculate liquid cultures for mating or for preparing competent cells. Seal the verified working stock plate with Parafilm and store at 4°C.

TABLE II. MATCHMAKER YEAST STRAIN PHENOTYPES

Strain	SD/–Ade	SD/–Met	SD/–Trp	SD/–Leu	SD/–His	SD/–Ura	YPDA
AH109	–	+	–	–	–	+	+
Y187	–	–	–	–	–	+	+

Notes:

- AH109 and Y187 can grow on SD/–Leu/–Trp if functional *TRP1* and *LEU2* genes are introduced.
- AH109 and AH109/Y187 diploids can grow on SD/–Ade/–His if the *ADE2* and *HIS3* genes—carried by AH109—are activated (i.e., in the presence of GAL4).

Section 2.0 Cell Culture

Section 2.3 Established Cells

Immortalization of murine microglial cells by a v-*raf* / v-*myc* carrying retrovirus

E. Blasi[1], R. Barluzzi[1], V. Bocchini[2], R. Mazzolla[1] and F. Bistoni[1]

[1]Department of Experimental Medicine and Biochemical Sciences, Microbiology University of Perugia, Perugia, Italy

[2]Molecular and Cellular Biology Sections, University of Perugia, Perugia, Italy

Received 12 September 1989; revised 8 November 1989; accepted 8 November 1990. Available online 13 November 2002.

Abstract

A murine cell line (BV-2) has been generated by infecting primary microglial cell cultures with a -*raf*/v-*myc* oncogene carrying retrovirus (J2). BV-2 cells expressed nonspecific esterase activity, phagocytic ability and lacked peroxidase activity. Such cells secreted lysozyme and, following appropriate stimulation, also interleukin 1 and tumor necrosis factor. Furthermore, BV-2 cells exhibited spontaneous anti-*Canada* activity and acquired tumoricidal activity upon treatment with interferon-γ. Phenotypically, BV-2 cells resulted positive for MAC1 and MAC2 antigens, and negative for MAC3, glial fibrillary acidic protein (GFAP) and galactocerebroside (GC) antigens. Since BV-2 cells retain most of the morphological, phenotypical and functional properties described for freshly isolated microglial cells, we can conclude that J2 virus infection has resulted in the immortalization of active microglial cells.

J Neurosci Res. 1992 Apr;31(4):616-21.

An immortalized cell line expresses properties of activated microglial cells.

Bocchini V, Mazzolla R, Barluzzi R, Blasi E, Sick P, Kettenmann H.

Department of Experimental Medicine and Biochemical Sciences, University of Perugia, Italy.

Abstract

Murine cultured microglial cells were immortalized after infection with a v-raf/v-myc recombinant retrovirus. This immortalized cell line (BV-2) shares properties with body macrophages with respect to the antigen profile, their phagocytic capacity and antimicrobial activity. BV-2 cells are not constitutively able to kill tumor cells in vitro, but acquire antitumor activity following an increase in [Ca++]i. BV-2 cells, like microglial cells, are however, distinct from peripheral macrophages by their expression of inwardly rectifying K+ channels in concert with a lack in outwardly rectifying K+ channels and the formation of spineous processes. The BV-2 cell line thus represents a suitable model for in vitro studies of activated microglial cells.

PMID: 1578513 [PubMed - indexed for MEDLINE]

Mouse Motor Neuron (NSC-34) Cell Line Maintenance

Catalogue #: CLU140

Description:

NSC-34 is a hybrid cell line, produced by fusion of motor neuron enriched, embryonic mouse spinal cord cells with mouse neuroblastoma. Cultures contain two populations of cells: small, undifferentiated cells that have the capacity to undergo cell division and larger, multi-nucleate cells. These cells express many properties of motor neurons, including choline acetyltransferase, acetylcholine synthesis, storage and release and neurofilament triplet proteins.

Applications: NSC-34 cells have been evaluate following exposure of cultures to a selection of chemicals know to be neurotoxic to motor neurons. NSC-34 cells respond to agents that affect voltage-gated ion channels, cytoskeletal organization and axonal transport. The sensitivity of action potential production to various ion channel blockers is similar to that in primary motor neurons in culture. Therefore, these immortalized motor neuron-like cells have the utility as a model for the investigation of neurotoxicity.

Cell culture conditions:

Media:
The cells grow in the high glucose formulation of DMEM supplemented with 10% FBS. Although not an absolute requirement, the cells can tolerate Pep/Strep used at 1X. Glutamine an also be added at 2-7mM.

Recommend media requirements:

DMEM: Sigma D5796 (with 4500 mg/L glucose, L-glutamine (0.584 g/L), sodium bicarbonate (3.7 g/L) without sodium pyruvate)
Fetal Bovine Serum, Qualified (US): PAA A15-751
Penicillin/Streptomycin, Liquid: Biochrome AG, A2213, contains 10,000 units of penicillin and 10,000 µg of streptomycin/ml

Thawing:
The cryovial is removed from liquid nitrogen and thawed quickly in a 37°C water bath. The cells are initially incubated in a 60 mm tissue culture plate in growth medium, as described above. The cells can be diluted 5-10 fold for the initial plating. It is not recommended to centrifuge the cells before plating, although it is known to be done. It has been found that the DMSO is less of a problem than the sensitivity of the cells immediately upon thawing.

***The same day, after the cells have attached to the plate (approximately 4-6 h), the medium should be refreshed to remove the DMSO. (If this procedure is not followed and the DMSO is removed the following day, the cells will likely be dead.)

Culture:
The cells grow right on the surface of the conventional tissue culture plastic; no special coating in required. Cell density is not a concern for the first few days after thawing because it is viewed more as a recovery period. Once they're growing properly then they can be plated at whatever density is most appropriate for the experiment being done.

Medium is typically changed every 2-3 days, depending on rate of growth. Cultures should be split at ~80% confluency 1:3-1:4. Remove cells from plate/flask using 0.25% trypsin-EDTA solution (~2 ml for 60-100 mm plate or T25 flask, 3 min at 37°C) and dilute 10X right away with DMEM-10.

Freezing:
It is highly recommended to freeze a few aliquots of the cells immediately after the initial growth/split to avoid losing the cell line.

The freezing medium recommended is as follows: 60% growth medium described above, 30% FBS supplemented with 10% sterile dimethylsulfoxide (DMSO) however variations of freezing media are acceptable. Target concentration of cells is 10^5/ml of freezing medium. Typically a confluent 100 mm plate or 50 ml culture flask will yield 6-10 cryovials.

The cells can be removed from the plate by trituration or using trypsin (as described above). The cells are centrifuged and the supernatant is removed. The cell pellet is then re-suspended and the small amount of liquid retained by surface tension. If this step is missed, the cells may clump when the larger volume of freezing medium is added. After re-suspending, 1.0ml of freezing medium is added and cells are place at -80C freezer or, better, in liquid N2 Cryogenic vials are placed in a NALGENETM Cryo 1°C Freezing Container overnight in a -80°C freezer. The next day the vials are transferred to a liquid nitrogen tank. It is recommended to test the cells for regrowth after freezing to be sure that the freezing procedure was performed correctly.

References:

He, B.P., Wen, W., and Strong, M. 2002. Activated microglia (BV-2) facilitation of TNF-α- mediated motor neuron death in vitro. *Journal of Neuroimmunology*. 128: 31-38.
Usuki S, Ren J, Utsunomiya I, Cashman NR, Inokuchi J, Miyatake T. 2001. GM2 ganglioside regulates the function of ciliary neurotrophic factor receptor in murine immortalized motor neuron-like cells (NSC-34). *Neurochem Res.* 2001 Apr;26(4):375-82.

Usuki S, Cashman NR, Miyatake T. 1999. GM2 promotes ciliary neurotrophic factor-dependent rescue of immortalized motor neuron-like cell (NSC-34). *Neurochem Res.* 1999 Feb;24(2):281-6.

Matsumoto A, Yoshino H, Yuki N, Hara Y, Cashman NR, Handa S, Miyatake T. 1995. Ganglioside characterization of a cell line displaying motor neuron-like phenotype: GM2 as a possible major ganglioside in motor neurons. *J Neurol Sci.* 1995 Aug;131(2):111-8.

Durham HD, Dahrouge S, Cashman NR. 1993. Evaluation of the spinal cord neuron X neuroblastoma hybrid cell line NSC-34 as a model for neurotoxicity testing. *Neurotoxicology.* 1993 Winter;14(4):387-95.

Cashman NR, Durham HD, Blusztajn JK, Oda K, Tabira T, Shaw IT, Dahrouge S, Antel JP. 1992. Neuroblastoma x spinal cord (NSC) hybrid cell lines resemble developing motor neurons. *Dev Dyn.* 1992 Jul;194(3):209-21

Cell Biology

ATCC® Number:	**CRL-11268™** Order this Item	Price:	**$272.00**
Designations:	293T/17 [HEK 293T/17]		
Depositors:	Rockefeller Univ.		
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	epithelial		
Source:	**Organ:** kidney		
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Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCC by Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.		
Antigen Expression:	SV40 T antigen [45408]		
Age:	fetus		

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Product Information Sheet

Comments:

The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCC CRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCC CRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCC CRL-11554) amphotropic envelope-expression packaging cell line.

Propagation:

ATCC complete growth medium: The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.
Temperature: 37.0°C
Atmosphere: air, 95%; carbon dioxide (CO2), 5%

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 1:4 to 1:8 is recommended
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
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 derivative:ATCC CRL-11269
References:	45408: Sena-Esteves M, et al. Single-step conversion of cells to retrovirus vector producers with herpes simplex virus-Epstein-Barr virus hybrid amplicons. J. Virol. 73: 10426-10439, 1999. PubMed: 10559361 57446: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 5,952,225 dated Sep 14 1999 57447: Pensiero M, et al. Retroviral vectors produced by producer cell lines resistant to lysis by human serum. US Patent 6,329,199 dated Dec 11 2001 57448: Pear WS, et al. Production of High-Titer Helper-Free Retroviruses by Transient Transfection. Proc. Natl. Acad. Sci. USA 90: 8392-8396, 1993. PubMed: 7690960

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Cell Biology

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Designations:	LADMAC		
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Shipped:	frozen		
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Growth Properties:	suspension, with some loosely adherent cells		
Organism:	*Mus musculus* (mouse)		
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Source:	**Organ:** bone marrow **Strain:** C3H		
Cellular Products:	colony stimulating factor-1 (CSF-1)		
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Comments:	LADMAC is a transformed cell line derived by transfecting mouse bone marrow cells highly enriched for macrophage progenitors with cloned human cellular myc-homologous sequences covalently attached to pBR325 (pR myc). The cell line has monocyte-like morphology; contains nonspecific esterase; is phagocytic for latex beads; secretes lysozyme, and bears the Mac-1 antigen. A minority of cells are Fc receptor positive and an appreciable number of cells are complement receptor 1 positive. The cells are tumorigenic in nu+, nu+ mice but not in syngenic mice. The cells are not phagocytic for antibody or complement-coated particles; they do not constitutively secrete Interleukin-1. LADMAC cells secrete the growth factor colony stimulating factor 1 (CSF-1). CSF-1 is capable of supporting the in vitro proliferation of mouse bone marrow macrophages. [38883] The Pannell-Milstein roller bottle apparatus may be used to produce high concentrations of CSF-1. [38884] This cell line is used to produce LADMAC conditioned medium. It will support the growth of the macrophage cell lines EOC 2 (CRL-2467), EOC 13.31 (CRL-2468), EOC 20 (CRL-2469), I-11.15 (CRL-2470) and I-13.35 (CRL-2471).
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%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 to 2 X 10 exp5 viable cells/ml. Maintain cell density between 1 X 10 exp5 and 1 X 10 exp6 viable cells/ml. Attached cells may be subcultured by tapping the sides of the flask until cells are dispersed. **Medium Renewal:** Add fresh medium every 2 to 3 days (depending on cell density)
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020

References:

38883: Sklar MD, et al. Transformation of mouse bone marrow cells by transfection with a human oncogene related to c-myc is associated with the endogenous production of macrophage colony stimulating factor 1. J. Cell. Physiol. 125: 403-412, 1985. PubMed: 3877730

38884: Olivas E, et al. Use of the Pannell-Milstein roller bottle apparatus to produce high concentrations of the CSF-1, the mouse macrophage growth factor. J. Immunol. Methods 182: 73-79, 1995. PubMed: 7769247

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Cell Biology

ATCC® Number:	**CRL-1721™** Order this Item	Price:	**$256.00**

Designations:	PC-12
Depositors:	B Patterson
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	floating clusters; few scattered lightly attached cells.
Organism:	Rattus norvegicus (rat)
Morphology:	small irregularly shaped cells 
Source:	**Organ:** adrenal gland **Disease:** pheochromocytoma
Cellular Products:	catecholamines; dopamine; norepinephrine [1163]
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.
Applications:	transfection host (Roche FuGENE® Transfection Reagents technology from amaxa)
Receptors:	nerve growth factor (NGF), expressed
Tumorigenic:	Yes
Cytogenetic Analysis:	40 chromosomes; 38 autosomes plus XY [1163]
Gender:	male
Comments:	The PC-12 cell line was derived from a transplantable rat pheochromocytoma. [1163] The cells respond reversibly to NGF by induction of the neuronal phenotype when plated on Collagen IV coated culture flasks. [1163] The cells do not synthesize epinephrine. [1163]

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Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. To make the complete growth medium, add the following components to the base medium: • heat-inactivated horse serum to a final concentration of 10% • fetal bovine serum to a final concentration of 5% **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol**: Volumes used for this protocol are for a 75cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. • Transfer cell suspension to centrifuge tube. Centrifuge cells at 180 to 225 xg for 8-15 minutes at room temperature. • Remove and discard supernatant leaving cell pellet. • Resuspend the cell pellet in an appropriate volume of fresh medium (about one tenth of the original volume. • Gently aspirate each 5 ml aliquot of cells 4 or 5 times with a new 20 ml syringe outfitted with a 22g (1½ in.) needle to break up cell clusters. • Add appropriate aliquots of the cell suspension to new 75 cm2 flask with 10-15 ml fresh growth medium. Seed flask 5 x 10(5) to 1 x 10(6) viable cells/ml or use subcultivation ratio of 1:2 to 1:4. • Place culture vessels in incubator at 37°C Subculture when cell density reaches between 2-4 x 10(6) viable cells/ml.
Preservation:	**Medium Renewal:** Every 2 to 3 days **Freeze medium:** Complete growth medium supplemented with 10% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase
Doubling Time:	48 hrs
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2001 recommended serum:ATCC 30-2020 ATCC CRL-1721.1, PC-1

References:

1162: Levi A, et al. Molecular cloning of a gene sequence regulated by nerve growth factor. Science 229: 393-395, 1985. PubMed: 3839317

1163: Greene LA, Tischler AS. Establishment of a noradrenergic clonal line of rat adrenal pheochromocytoma cells which respond to nerve growth factor. Proc. Natl. Acad. Sci. USA 73: 2424-2428, 1976. PubMed: 1065897

22344: Biocca S, et al. A macromolecular structure favouring microtubule assembly in NGF- differentiated pheochromocytoma cells (PC12). EMBO J. 2: 643-648, 1983. PubMed: 6641712

33014: Weber E, et al. Distinct functional properties of Rab3A and Rab3B in PC12 neuroendocrine cells. J. Biol. Chem. 271: 6963-6971, 1996. PubMed: 8636125

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ATCC® Number:	**CCL-131™** Order this Item	Price:	**$256.00**
Designations:	Neuro-2a		
Depositors:	RJ Klebe		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Mus musculus* (mouse)		
Morphology:	neuronal and amoeboid stem cells 		
Source:	**Strain:** A **Organ:** brain **Disease:** neuroblastoma **Cell Type:** neuroblast;		
Cellular Products:	acetylcholinesterase tubulin		
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.		
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)		
Virus Susceptibility:	Herpes simplex virus Vesicular stomatitis virus Human poliovirus 1		
Antigen Expression:	H-2, a haplotype; Mus musculus, expressed		
Cytogenetic Analysis:	modal number = 95; range = 59 to 193. Karyotype unstable within a stemline range of 94 to 98 chromosomes. All the cells contain 6 to 10 large chromosomes with median or submedian centromeres and 2 to 4 minute chromosomes.		
GenoType:	albino		

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Comments:	Clone Neuro-2a was established by R.J. Klebe and F.H. Ruddle from a spontaneous tumor of a strain A albino mouse. This tumor line, designated C1300, was obtained from the Jackson Laboratory, Bar Harbor, Maine [22161].Neuro-2a cells produce large quantities of microtubular protein which is believed to play a role in a contractile system which is responsible for axoplasmic flow in nerve cells.The cell line has been used for studies on the mechanism of vinblastine precipitation of microtubular protein, the kinetics of GTP binding to isolated protein, the turnover of microtubules in vivo, and the synthesis and assembly of microtubular protein [PubMed: 5263744]. The World Organization for Animal Health (OIE) uses the cells for routine diagnosis of rabies. (see: http://www.oie.int /Eng/Normes/Mmanual/A_00044.htm)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: fetal bovine 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 37C 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 37C.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** 1 to 2 times per week **Freeze medium:** Complete growth medium, 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor phase

Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020 0.25% (w/v) Trypsin - 0.53 mM EDTA in Hank' BSS (w/o Ca++, Mg++):ATCC 30-2101 Cell culture tested DMSO:ATCC 4-X
References:	1023: Olmsted JB, et al. Isolation of microtubule protein from cultured mouse neuroblastoma cells. Proc. Natl. Acad. Sci. USA 65: 129-136, 1970. PubMed: 5263744 22161: Klebe RJ, Ruddle FH. Neuroblastoma: Cell culture analysis of a differentiating stem cell system. J. Cell Biol. 43: 69A, 1969. 29352: Naslavsky N, et al. Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J. Biol. Chem. 272: 6324-6331, 1997. PubMed: 9045652 29861: Kaneko K, et al. Evidence for protein X binding to a discontinuous epitope on the cellular prion protein during scrapie prion propagation. Proc. Natl. Acad. Sci. USA 94: 10069-10074, 1997. PubMed: 9294164 32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

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Cell Biology

ATCC® Number:	**CRL-2469™** Order this Item	Price:	**$438.00**

Designations:	EOC 20
Depositors:	WS Walker
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	macrophage
Source:	**Organ:** brain **Strain:** C3H/HeJ **Cell Type:** microglia;
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.
Receptors:	colony stimulating factor 1 (CSF-1R, CD115)
Antigen Expression:	CD11b/CD18 (Mac-1) +, Mac-2 +, Mac-3 +, CD80 (B7-1) +, CD45 +, Ly-6C +, MHC Class I +, MHC Class II +, CD115 (colony stimulating factor 1 receptor (CSF-1R)) +, FcR +, F4/80 +/-, CD86 (B7.2) - [39974]
Age:	10 days juvenile
Gender:	female
Comments:	This is an immortalized cell line derived from the brain of an apparently normal 10 day old mouse [PubMed: 8550814]. Cells were cloned in soft agar in the presence of CSF-1 and expanded on microcarrier beads. Beads were transferred to culture dishes and were subsequently passaged by scraping. The cell line is dependent on growth factor colony stimulating factor 1 (CSF-1). Conditioned medium is made from LADMAC cells (ATCC CRL-2420?) as a source of CSF-1.The cells exhibit phagocytic activity. These cells constitutively expressed high levels of major histocompatibility complex (MHC) class II antigens and expression was upregulated by recombinant murine interferon-gamma. The cells may be used to characterize the role of brain macrophages in immune responses in the central nervous system (CNS).

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Propagation:	**ATCC complete growth medium:** Dulbecco's modified Eagle's medium with 4 mM L-glutamine adjusted to contain 1.5 g/L sodium bicarbonate and 4.5 g/L glucose, 70%; fetal bovine serum, 10%; LADMAC Conditioned Media (produced from the LADMAC cell line (CRL-2420), 20% **Temperature:** 37.0°C
Subculturing:	**Subcultivation Ratio:** A subcultivation ratio of 1:3 is recommended **Medium Renewal:** Every 2 to 3 days Remove 75% of the media. Scrape off the attached cells and transfer into new flasks.
Preservation:	culture medium 95%; DMSO, 5%
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002 recommended serum:ATCC 30-2020 purified RNA:ATCC CRL-2469R
References:	38884: Olivas E, et al. Use of the Pannell-Milstein roller bottle apparatus to produce high concentrations of the CSF-1, the mouse macrophage growth factor. J. Immunol. Methods 182: 73-79, 1995. PubMed: 7769247 39968: Walker WS. Establishment of mononuclear phagocyte cell lines. J. Immunol. Methods 174: 25-31, 1994. PubMed: 8083530 39974: Walker WS, et al. Mouse microglial cell lines differing in constitutive and interferon-gamma-inducible antigen-presenting activities for naive and memory CD4+ and CD8+ T cells. J. Neuroimmunol. 63: 163-174, 1995. PubMed: 8550814 39975: Askew D, Walker WS. Alloantigen presentation to naive CD8+ T cells by mouse microglia: evidence for a distinct phenotype based on expression of surface-associated and soluble costimulatory molecules. Glia 18: 118-128, 1996. PubMed: 8913775

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Cell Biology

ATCC® Number:	**CRL-10442™** Order this Item	Price:	**$324.00**
Designations:	HCN-1A		
Depositors:	Johns Hopkins University		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	neuronal 		
Source:	**Organ:** brain **Cell Type:** cortical neuron;		
Cellular Products:	tubulin; neurofilament protein; somatostatin; cholecystokinin-8		
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.		
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11,12 D16S539: 12 D5S818: 11,12 D7S820: 11,12 THO1: 9.3 TPOX: 11 vWA: 17		
Age:	18 months		
Gender:	female		

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	The cells stain positively for a number of neuronal markers including neurofilament protein, neuron specific enolase (NSE). [48286] They are also positive for tubulin, vimentin, somatostatin (SST), glutamate, gamma aminobutyric acid (GABA), cholecystokinin - 8 (CCK-8) and vasoactive intestinal peptide (VIP). [22022] The cells are negative for glial fibrillary acidic protein (GFAP) and myelin basis protein (MBP). [48286]
Comments:	HCN-1A cells can be induced to differentiate when cultured with a mixture of nerve growth factor (NGF), dibutyryl cyclic adenosine monophosphate (cAMP) and 1-isobutyl-3-methylxanthine (IBMX). [22022] Differentiation is accompanied by mature morphology and slowing of growth (doubling time greater than 120 hours). [22022] Unlike HCN-2 (see ATCC CRL-10742) the growth rate of HCN-1A cells is not affected by phorbol esters. [22022]
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Growth Conditions:** The growth medium must be adjusted to pH 7.35 prior to filtration

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 which 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 37C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. To remove trypsin-EDTA solution, transfer cell suspension to centrifuge tube and spin at approximately 125 xg for 5 to 10 minutes.
6. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels.
7. Place culture vessels in incubators at 37C.

CRL-10442 has been shown to senesce at approximately passage 19. Current distribution stocks are prepared with a minimum of only 2 passages remaining under recommended culture conditions after cryopreservation.
Subcultivation Ratio: A subcultivation ratio of 1:2 to 1:3 is recommended
Medium Renewal: 1 to 2 times per week

Preservation:

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

Related Products:

Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2002
recommended serum:ATCC 30-2020

References:

22022: Ronnett GV, et al. Human neuronal cell line. US Patent 5,196,315 dated Mar 23 1993
48286: Ronnett GV, et al. Human cortical neuronal cell line: establishment from a patient with unilateral megalencephaly. Science 248: 603-605, 1990. PubMed: 1692158

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ATCC® Number:	**CCL-127™** Order this Item	Price:	**$264.00**
Designations:	IMR-32		
Depositors:	WW Nichols		
Biosafety Level:	1		
Shipped:	frozen		
Medium & Serum:	See Propagation		
Growth Properties:	adherent		
Organism:	*Homo sapiens* (human)		
Morphology:	fibroblast; neuroblast 		
Source:	**Organ:** brain **Disease:** neuroblastoma **Cell Type:** neuroblast;		
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.		
Isolation:	**Isolation date:** April, 1967		
Applications:	transfection host (technology from amaxa)		
Virus Resistance:	echovirus 11		
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 11,12 D13S317: 9 D16S539: 8 D5S818: 11,12 D7S820: 9,10 THO1: 7,9.3 TPOX: 11 vWA: 15		
Cytogenetic Analysis:	Stable male karyotype with stemline number of 49. Two large marker chromosomes with submedian centromeres. A deletion in one number 1 chromosome: One number 16 chromosome missing; two extra chromosomes in C group. Sublines with 50 and 48 chromosomes differ from those with 49 chromosomes by having an extra or missing C group chromosome respectively.		
Isoenzymes:	G6PD, B		
Age:	13 months		

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Gender:	male
Ethnicity:	Caucasian
Comments:	The IMR-32 cell line was established by W.W. Nichols, J. Lee and S. Dwight in April, 1967 from an abdominal mass occurring in a 13-month-old Caucasian male. [22190] The tumor was diagnosed as a neuroblastoma with rare areas of organoid differentiation. Two cell types are present. Predominant is a small neuroblast-like cell. The other is a large hyaline fibroblast. The cell line was submitted to the American Type Culture Collection in the 36th passage. It has been demonstrated that the cells can be propagated successfully beyond the 80th serial subculture.
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%. **Temperature:** 37.0°C
Subculturing:	**Protocol:** Remove medium, and rinse with 0.25% trypsin, 0.53 mM EDTA solution. Remove the solution and add an additional 1 to 2 ml of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks.Maintain cultures at a cell concentration between 4 X 10 exp4 and 4 X 10 exp5 cells/cm2. **Subcultivation Ratio:** A subcultivation ratio of 1:3 to 1:6 is recommended **Medium Renewal:** Every 2 to 3 days
Preservation:	**Freeze medium:** Complete growth medium 95%; DMSO, 5% **Storage temperature:** liquid nitrogen vapor temperature
Doubling Time:	approximately 20 hrs.
Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 recommended serum:ATCC 30-2020
References:	22190: Tumilowicz JJ, et al. Definition of a continuous human cell line derived from neuroblastoma. Cancer Res. 30: 2110-2118, 1970. PubMed: 5459762 32287: Rostomily RC, et al. Expression of neurogenic basic helix-loop-helix genes in primitive neuroectodermal tumors. Cancer Res. 57: 3526-3531, 1997. PubMed: 9270024 32459: Maestrini E, et al. A family of transmembrane proteins with homology to the MET-hepatocyte growth factor receptor. Proc. Natl. Acad. Sci. USA 93: 674-678, 1996. PubMed: 8570614

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ATCC® Number:	**CCL-1™** Order this Item	Price:	**$256.00**

Designations:	NCTC clone 929 [L cell, L-929, derivative of Strain L]
Depositors:	WR Earle
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
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 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.
Isolation:	**Isolation date:** March, 1948
Applications:	testing [92346] [92380] [92382] [92389] [92404] toxicity testing [21469] [21470] [21606] transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Resistance:	poliovirus 1, 2, 3; coxsackievirus B5; polyomavirus
Tumorigenic:	Yes
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

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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. [25770] Clone 929 was established (by the capillary technique for single cell isolation) from the 95th subculture generation of the parent strain. [21404] 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.
Preservation:	**Subcultivation Ratio:** A subcultivation ratio of 1:2 to 1:8 is recommended **Medium Renewal:** 2 to 3 times per week **Freeze medium:** Complete growth medium supplemented with 5% (v/v) DMSO **Storage temperature:** liquid nitrogen vapor phase

Related Products:	Recommended medium (without the additional supplements or serum described under ATCC Medium):ATCC 30-2003 derivative:ATCC CCL-1.1 derivative:ATCC CCL-1.2 derivative:ATCC CCL-1.3 derivative:ATCC CCL-1.4

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: 6325324
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.
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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: 13233820
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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: 13293658
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29223: Papkoff J. Regulation of complexed and free catenin pools by distinct mechanisms. J. Biol. Chem. 272: 4536-4543, 1997. PubMed: 9020180
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

Section 4.0 Genetically Modified Organisms and Cell Lines

Section 4.2 Appendix B: Plasmids

Plasmid	Notes	Supplier
pAS2-1	bait protein for Y2H	Clontech #K1604-B
pBluescript SK(-)	cloning with blue/white screening, T7, T3	Agilent
pBridge	yeast, two MCS's	Clontech #630404
pcDNA3.1(+)	mammalian expression, T7	Invitrogen #V790-20
pcDNA3.1/myc-HisA	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pcDNA3.1/myc-HisB	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pcDNA3.1/myc-HisC	mammalian expression,T7, Myc/His tags	Invitrogen #V800-20
pCMV-SPORT6	generation of cDNA libraries	Invitrogen #12209-011
pCMX	constituative mammalian expression	unknown
pCRII-TOPO	cloning, Sp6, T7	Invitrogen K4650-01
pCR-XL-TOPO	cloning of long PCR products	Invitrogen K4750-10
pECFP-C1	fusion protein at C-terminus of CFP @475nm	Clontech #6076-1
pDsRed1-N1	fusion protein at N-terminus of RFP @582nm	Clontech 6921-1
pEGFP-C1	fusion protein at C-terminus of GFP @507nm	Clontech #6084-1
pEGFP-N1	fusion protein at N-terminus of GFP @507nm	Clontech #6085-1
pEYFP-C1	fusion protein at C-terminus of YFP @527nm	Clontech #6005-1
pEYFP-N1	fusion protein at N-terminus of YFP @527nm	Clontech #6006-1
pGAD-424	yeast shuttle vector	Clontech #K1605-1
pGAD-T7	yeast shuttle vector + HA tag, T7	Clontech #K1612-1
pGAD-T7-Rec	cDNA library construction for Y2H	Clontech #630490
pGBKT7-53	positive control for Y2H	Clontec #630445
pGBKT7-DNA-BD	GAL4 DNA-BD+Myc+Fusion protein	Clontech #630443
pGBKT7-LAM	negative control for bkgd inY2H	Clontech #630445
pGEM-4Z	cloning with blue/white screening, T7, SP6	Promega # P2161
pGEM-7Zf(+)	cloning, T7, Sp6	Promega #P2251
pGEM-7Zf(-)	cloning, T7, Sp6	Promega #P2371
pGEM-T Easy	cloning with blue/white screening, T7, SP6	Promega #E1360
pGEX-2T	GST-fusion/thrombin cleavage	GE Healthcare 28-9546-53
pGEX-4T2	GST-fusion/thrombin cleavage	GE Healthcare 28-9545-50
pGEX5X-3	GST-fusion/factor Xa cleavage	GE Healthcare 28-9545-55
pGL3-Control	luciferase reporter vector	Promega E1741
pIRES-EGFP	fusion+IRES +YFP	Clontech #6064-1
pMIR GLO	Dual-Luciferase miRNA Target Expression	Promega #E1330
pOTB7	cloning, CAM^R, T7, SP6	unknown
pRFP-N1	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRFP-N2	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRFP-N3	fusion protein at N-terminus of RFP @582nm	Clontech (discontinued)
pRK172	cloning, T7	Dr DP Hanger
pRSVi	cloning	Dr R. Liem
pSuper	endogenous production of siRNA	Oligoengine VEC-PBS-0001/0002

Plasmid	Notes	Supplier
pT7T3D	cloning, T3, T7	Image
pTET-Off Advanced	to develop stableTet-Off cell lines	Clontech #631126
pTRE-Tight	response plasmid expressing gene	Clontech #631126
pTRE Tight Luc	expresses luciferase	Clontech #631126
pTRIamp19	T3, T7 and Sp6 promotors	Ambion 7424

III. GAL4 DNA-Binding Domain (DNA-BD) Cloning Vectors *continued*

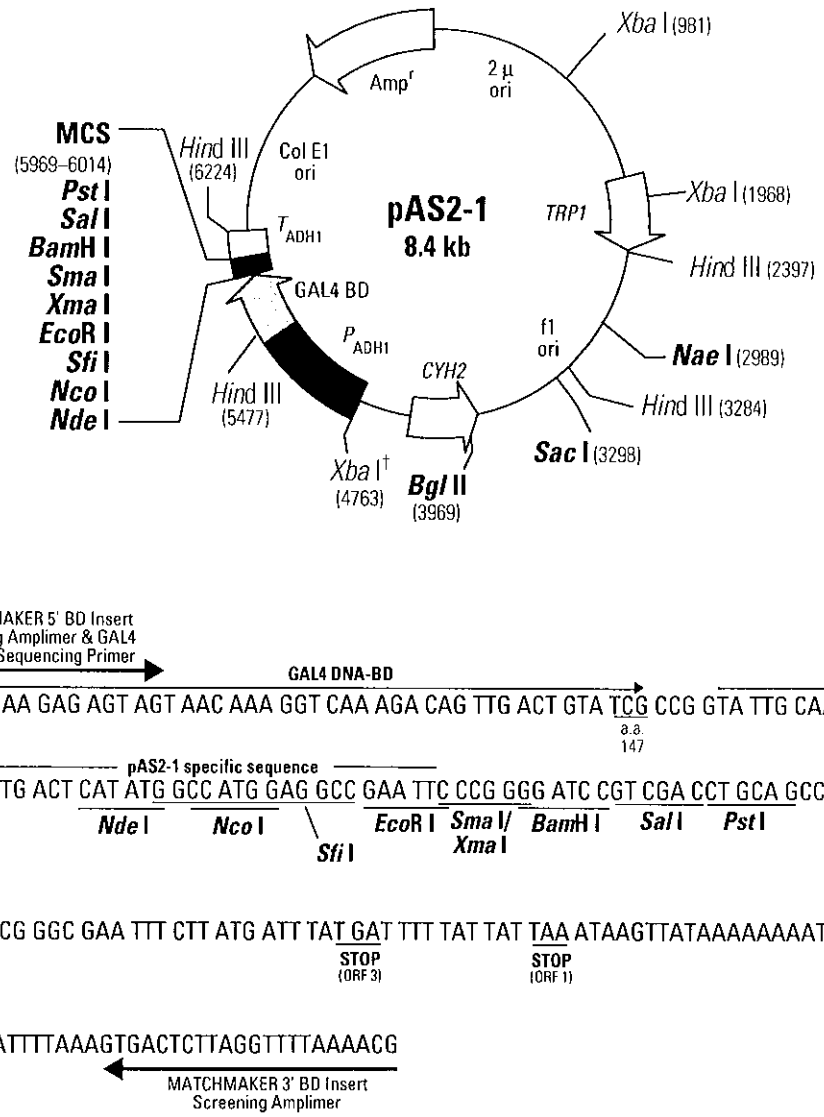
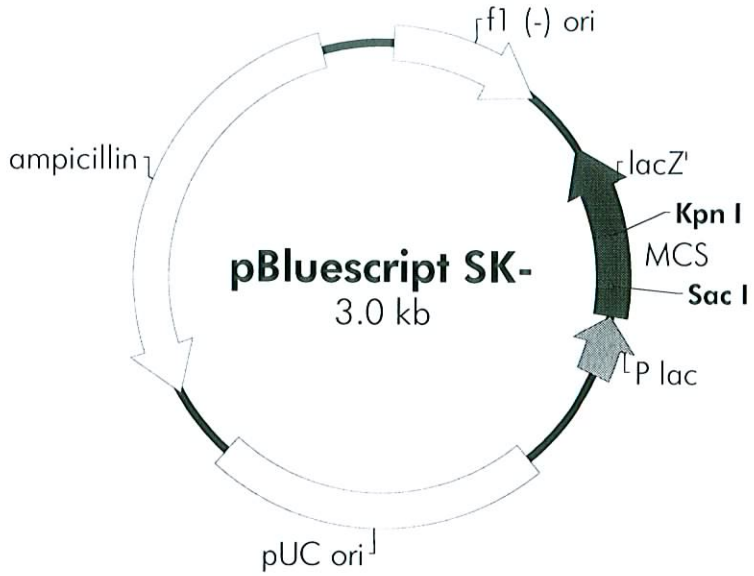


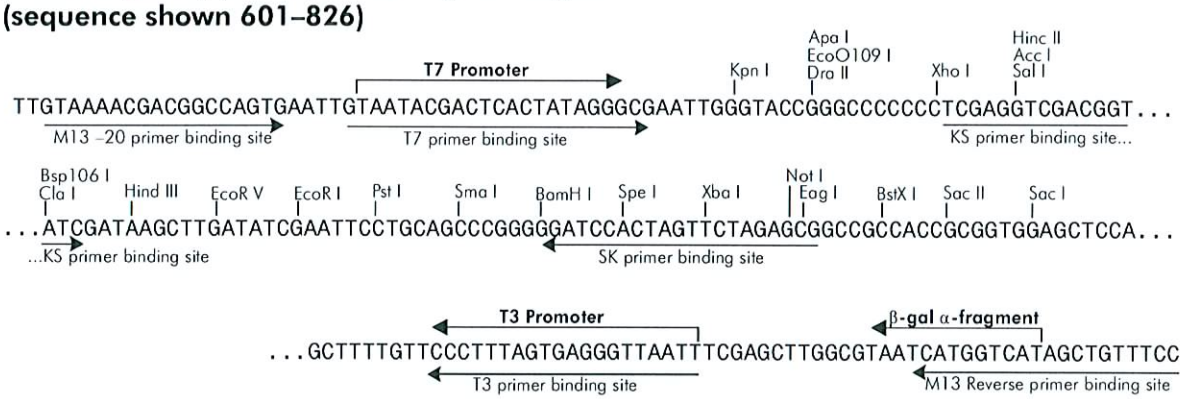
Figure 9. pAS2-1 map and MCS. Unique sites are in bold. pAS2-1 is a cloning vector used to generate fusions of a bait protein with the GAL4 DNA-BD (a.a. 1–147). pAS2-1 is derived from pAS2 (Figure 8) and hence from $pAS1_{CHY2}$ (Harper *et al.*, 1993) and carries the CYH^S2 gene for cycloheximide sensitivity. The hybrid protein is expressed at high levels in yeast host cells from the full-length *ADH1* promoter (P_{ADH1}). The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences (Silver *et al.*, 1984). The *Xba* I site at bp 4763 (†) is methylation sensitive. pAS2-1 contains the *TRP1* gene for selection in Trp^- auxotrophic yeast strains. Plasmid modification was performed at CLONTECH. GenBank Accession: #U30497.

Compared with pAS2, pAS2-1 contains a neutral, short peptide instead of an HA epitope tag. Removing the HA epitope tag and converting a.a.149 from Glu to Val completely eliminates the autonomous activation activity of pAS2 (assayed in Y187 using the *lacZ* reporter; Holtz & Zhu, 1995). pAS2-1 has a different MCS than pAS2; however, the cloning sites that they have in common are in the same reading frame. Furthermore, the *Eco*R I, *Xma* I/*Sma* I, *Bam*H I, Sal I, and Pst I sites in the pAS2-1 MCS are in the same reading frames as those in pGBT9. Thus, inserts from pAS2 or pGBT9 can be excised and moved to pAS2-1 without changing the reading frame. The *Eco*R I site in the pAS2-1 MCS is unique because the *Eco*R I site in the CYH^S2 gene in pAS2 was eliminated by site-directed mutagenesis.

pBluescript SK(–) Vector Map



pBluescript SK (–) Multiple Cloning Site Region (sequence shown 601–826)



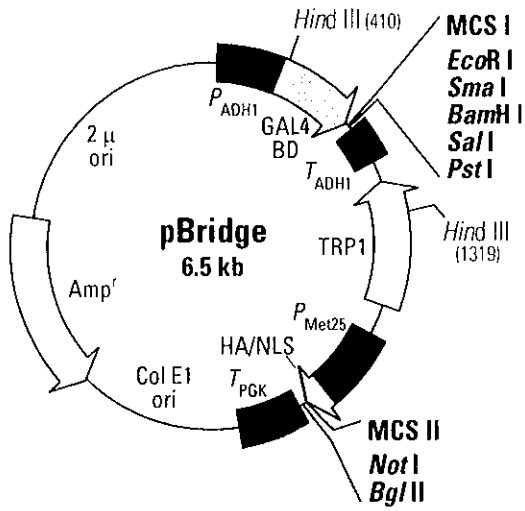
Feature	Nucleotide Position
f1 (–) origin of ss-DNA replication	24–330
β-galactosidase α-fragment coding sequence (lacZ')	463–816
T7 promoter transcription initiation site	643
multiple cloning site	653–760
T3 promoter transcription initiation site	774
lac promoter	817–938
pUC origin of replication	1158–1825
ampicillin resistance (*bla*) ORF	1976–2833

FIGURE 2 Circular map and polylinker sequence of the pBluescript SK(–) phagemid. The complete sequence and list of restriction sites are available from *www.stratagene.com* or from the GenBank® database (#X52324).

Vector Information

pBridge™ Vector Information PT3212-5

GenBank Accession No.: Submission in progress. Cat. No. 630404



MCS I:

MATCHMAKER 5' DNA-BD Insert Screening Amplimer & GAL4 DNA-BD Sequencing Primer

827 → GAL4 DNA-BD

TCA TCG GAA GAG AGT AGT AAC AAA GGT CAA AGA CAG TTG ACT GTA TCG CCG

878

GAA TTC CCG GGG ATC CGT CGA CCT GCA GCC AAG CTA ATT CCG GGC GAA TTT

EcoR I *Sma I* *BamH I* *Sal I* *Pst I* STOP (ORF 2)

929

CTT ATG ATT TAT GAT TTT TAT TAT TAA ATA AGT TAT AAA AAA AAT AAG TGT ATA

STOP (ORF 3) STOP (ORF 1)

983

CAA ATT TTA AAG TGA CTC TTA GGT TTT AAA ACG ←

MATCHMAKER 3' DNA-BD Insert Screening Amplimer

2682

MCS II: AAG AAG AGA AAG GTG GCG GCC GCA TTA GCC CGA AGA TCT TCG GGC TGA

Not I *Bgl II* STOP

Restriction map and multiple cloning sites of pBridge Vector. Unique restriction sites are in bold.

Description:

pBridge™ expresses two proteins: a DNA-binding domain fusion, and an additional protein (1–3). pBridge thus allows establishment of three-hybrid systems when used in combination with an activation domain fusion vector and yeast strains from any of Clontech's GAL4-based two-hybrid systems, including Matchmaker™ Gold. This vector generates a hybrid protein that contains the sequences for the GAL4 DNA-binding domain (DNA-BD; a.a. 1–147) and the sequence cloned into MCS I. The fusion protein is expressed in yeast host cells from the constitutive *ADH1* promoter; transcription is terminated at the *ADH1* transcription termination signal. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences (NLS) that are an intrinsic part of the GAL4 DNA-BD (3). An additional gene of interest can be cloned into MCS II which is located downstream of an HA epitope and a second NLS. The resulting fusion protein is conditionally expressed from the *MET25* promoter in response to methionine levels in the medium; i.e., it is repressed in the presence of 1 mM methionine and expressed in the absence of methionine (1).

(PR912673)

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A Takara Bio Company
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Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

pBridge is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae*. It carries the *bla* gene (for ampicillin resistance in *E. coli*) and the *TRP1* nutritional marker that allow yeast auxotrophs carrying pBridge to grow on limiting synthetic medium lacking tryptophan. **Note:** Yeast strain Y187 is a methionine auxotroph; therefore, haploid Y187 harboring pBridge cannot be grown on medium lacking methionine.

Use:

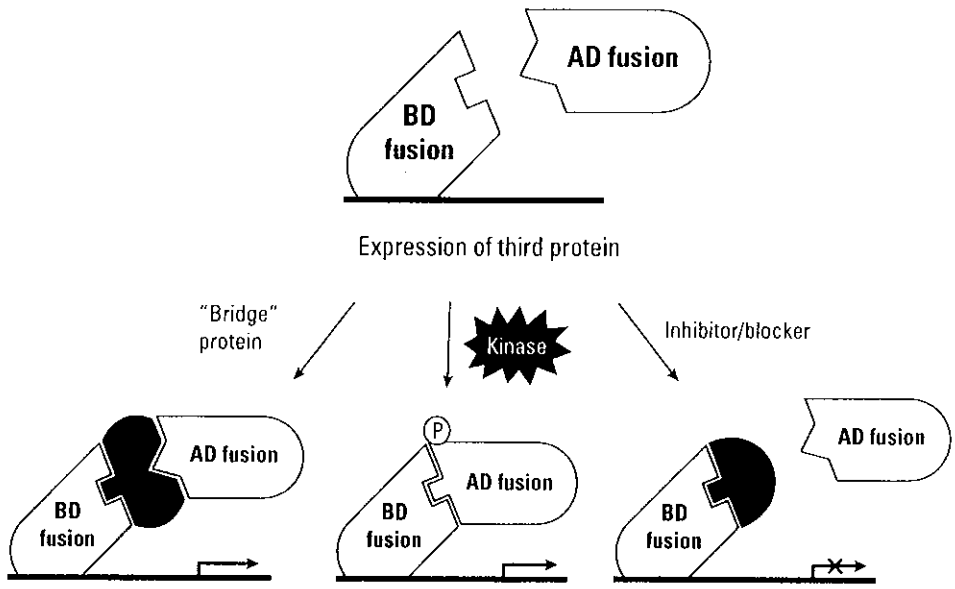
The pBridge Vector now makes it possible to investigate ternary protein complex formation (1, 2). pBridge contains two distinct multiple cloning sites to allow expression of the DNA-BD fusion as well as a third protein. When pBridge is used in conjunction with the AD fusion vector and yeast strains from any one of Clontech's GAL4-based two-hybrid systems, a 'three-hybrid' system can be established hat is dependent on the expression of a third protein.

The yeast two-hybrid system has proven to be a powerful molecular approach for detecting protein-protein (binary) interactions and has contributed significantly to the dissection of many molecular pathways. However, the *two*-hybrid system is designed to detect the interaction between just two proteins, which are expressed as the AD and BD fusions.

The figure below demonstrates the more complex protein interactions that can be investigated with the three-hybrid system. The third protein in this system can participate in the interaction in several ways: as a "bridge", interacting with two proteins that do not directly interact with each other; to stabilize a weak interaction between two proteins; or as an inhibitor or modifier (e.g., kinase; 3) of one or both of the proteins. Alternatively, a competitor of a two-hybrid interaction can be expressed from this promoter to confirm the specificity of the two-hybrid interaction (1). The conditional expression of the third protein allows investigation of its role in the interaction between the AD and BD fusion proteins. Like the two-hybrid system, the three-hybrid system can be used to screen libraries to clone new interacting partners, either for the third protein or the binding domain fusion.

Expression of the third protein is controlled by a conditional methionine promoter (P_{MET25}) such that it is expressed in the absence of methionine. This allows expression to be switched on or off by a simple replica plating step. The effect of the third protein is indicated by expression of a reporter or nutritional marker.

To facilitate experiments with pBridge, we offer three drop-out media supplements that lack methionine: –His/–Leu/–Met/–Trp (Cat No. 630429); –Leu–Met/–Trp (Cat. No. 630430); and –Met/–Trp (Cat. No. 630431).



The three-hybrid system. pBridge expresses both the DNA-BD fusion and the third protein. The activation domain fusion is expressed from a separate two-hybrid system vector. The conditionally expressed third protein can play a structural (left), modifying (center), or inhibitory (right) role in the interaction that restores reporter gene expression.

Location of features:

- Promoter
  Fragment containing the *S. cerevisiae ADH1* promoter: 10–406
- GAL4 DNA-binding domain polypeptide
  Start codon: 434–436; stop codon: 953–955
  GAL4 codons 1–147: 434–874
- MCS I: 878–905
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator: 921–1112
- *TRP1* coding sequence
  Start codon: 1835–1833; stop codon: 1163–1161
- *S. cerevisiae MET25* promoter: 2117–2609
- HA epitope and nuclear localization sequence: 2610–2699
- MCS II: 2700–2723
- *S. cerevisiae PGK* terminator: 2733–3120
- Col E1 origin of replication: 3324–3967
- Ampicillin resistance gene (β-lactamase): 5045–3968
  Promoter: 5045–5017
  Coding sequence: 4975–4115
- Fragment containing the 2 μ origin of replication: 5362–6526

Primer locations:

- Matchmaker DNA-BD 5' Insert Screening Amplimer (Cat. No. 5417-1) or GAL4 BD Sequencing Primer (Cat. No. 6474-1): 827—843
- Matchmaker DNA-BD 3' Insert Screening Amplimer (Cat. No. 5417-1): 1015–994

Propagation in *E. coli*:

- Suitable host strains: DH5α and other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: 15–20

Propagation in *S. cerevisiae*:

- Suitable host strains: Y187(α)*, Y190(a), HF7c(a), CG1945(a), PJ69-2A(a), PJ69-4A(a)*, SFY526(a), and AH109(a).
- Selectable marker: *TRP1*
- *S. cerevisiae* replication origin: 2 μ
- Copy number: multiple copy

References:

1. Tirode, F., *et al.* (1997) *J. Biol. Chem.* **272**:22995–22999.
2. Brachmann, R. & Boeke, J. (1997) *Curr. Opin. Biotechnol.* **8**:561–568.
3. Osborne, M., *et al.* (1995) *Biotechnology* **13**:1474–1478.

* These strains are methionine auxotrophs; protein cannot be expressed from MCS II of pBridge in these strains. However, these strains may be used when mating to a methionine autotroph.

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 Clontech. This vector has not been completely sequenced.

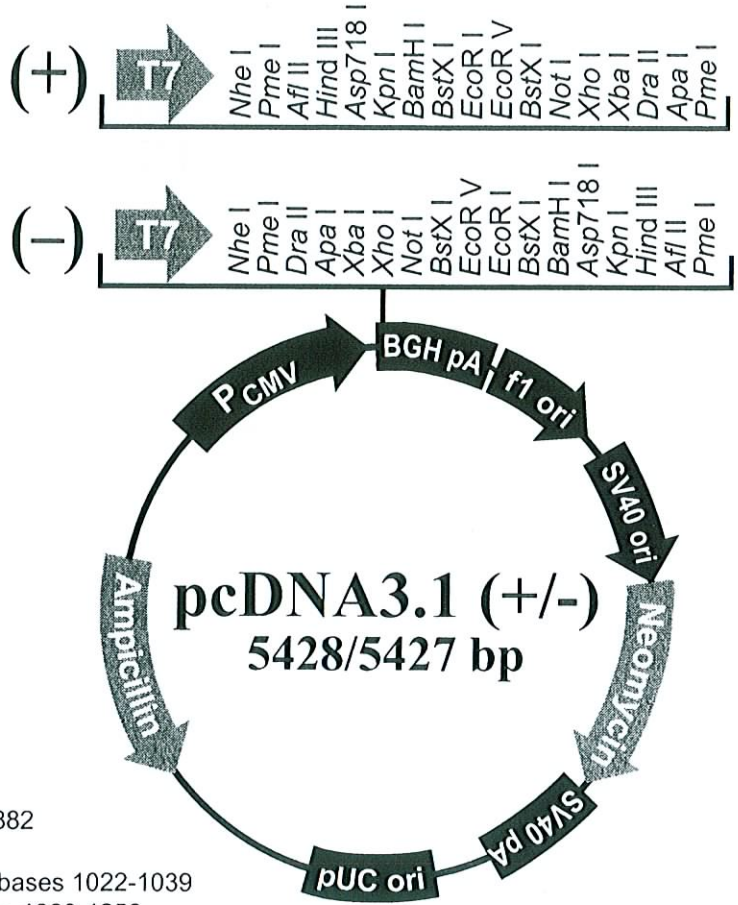
Appendix

pcDNA3.1 Vectors

Invitrogen #430018 (old)
#V790-20 (new)

Map of pcDNA3.1(+) and pcDNA3.1(-)

The figure below summarizes the features of the pcDNA3.1(+) and pcDNA3.1(-) vectors. **The complete sequences for pcDNA3.1(+) and pcDNA3.1(-) are available for downloading from our World Wide Web site** (www.invitrogen.com) **or from Technical Service (see page 13).** Details of the multiple cloning sites are shown on page 3 for pcDNA3.1(+) and page 4 for pcDNA3.1(-).



Comments for pcDNA3.1 (+)
5428 nucleotides

CMV promoter: bases 232-819
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 895-1010
pcDNA3.1/BGH reverse priming site: bases 1022-1039
BGH polyadenylation sequence: bases 1028-1252
f1 origin: bases 1298-1726
SV40 early promoter and origin: bases 1731-2074
Neomycin resistance gene (ORF): bases 2136-2930
SV40 early polyadenylation signal: bases 3104-3234
pUC origin: bases 3617-4287 (complementary strand)
Ampicillin resistance gene (*bla*): bases 4432-5428 (complementary strand)
ORF: bases 4432-5292 (complementary strand)
Ribosome binding site: bases 5300-5304 (complementary strand)
bla promoter (P3): bases 5327-5333 (complementary strand)

continued on next page

NB needs start ~~Stop~~ codons

Cloning into pcDNA3.1, continued

Multiple Cloning Site of pcDNA3.1(+)

Below is the multiple cloning site for pcDNA3.1(+). Restriction sites are labeled to indicate the cleavage site. The *Xba* I site contains an internal stop codon (TCTAGA). The multiple cloning site has been confirmed by sequencing and functional testing. **The complete sequence of pcDNA3.1(+) is available for downloading from our web site** (www.invitrogen.com) **or from Technical Service (see page 13).** For a map and a description of the features of pcDNA3.1(+), please refer to the **Appendix**, pages 10-11.

enhancer region (3′ end)

```
689  CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA CGGGACTTTC CAAAATGTCG
```

CAAT TATA

```
749  TAACAACTCC GCCCCATTGA CGCAAATGGG CGGTAGGCGT GTACGGTGGG AGGTCTATAT
```

3′ end of hCMV putative transcriptional start

```
809  AAGCAGAGCT CTCTGGCTAA CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC
```

T7 promoter/primer binding site *Nhe* I *Pme* I *Afl* II *Hind* III *Asp*718 I *Kpn* I

```
869  GACTCACTAT AGGGAGACCC AAGCTGGCTA GCGTTTAAAC TTAAGCTTGG TACCGAGCTC
```

*Bam*H I *Bst*X I* *Eco*R I *Eco*R V *Bst*X I* *Not* I *Xho* I

```
929  GGATCCACTA GTCCAGTGTG GTGGAATTCT GCAGATATCC AGCACAGTGG CGGCCGCTCG
```

Xba I *Apa* I *Pme* I pcDNA3.1/BGH reverse priming site

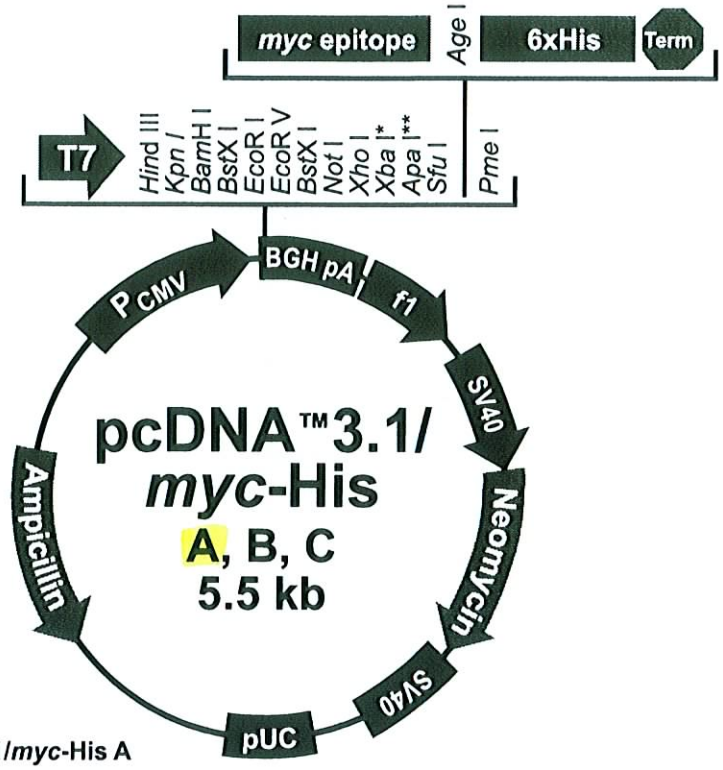
```
989  AGTCTAGAGG GCCCGTTTAA ACCCGCTGAT CAGCCTCGAC TGTGCCTTCT AGTTGCCAGC

1049 CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT GGAAGGTGCC ACTCCCACTG
```

BGH poly (A) site

```
1109 TCCTTTCCTA ATAAAATGAG GAAATTGCAT
```

*Please note that there are two *Bst*X I sites in the polylinker.

continued on next page



Comments for pcDNA™3.1/*myc*-His A
5493 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
Multiple cloning site: bases 902-999
myc epitope: bases 997-1026
Polyhistidine tag: bases 1042-1059
BGH reverse priming site: bases 1082-1099
BGH polyadenylation signal: bases 1081-1295
f1 origin of replication: bases 1358-1771
SV40 promoter and origin: bases 1836-2160
Neomycin resistance gene: bases 2196-2990
SV40 polyadenylation signal: bases 3166-3296
pUC origin: bases 3679-4352
Ampicillin resistance gene: bases 4497-5357 (complementary strand)

* There is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.

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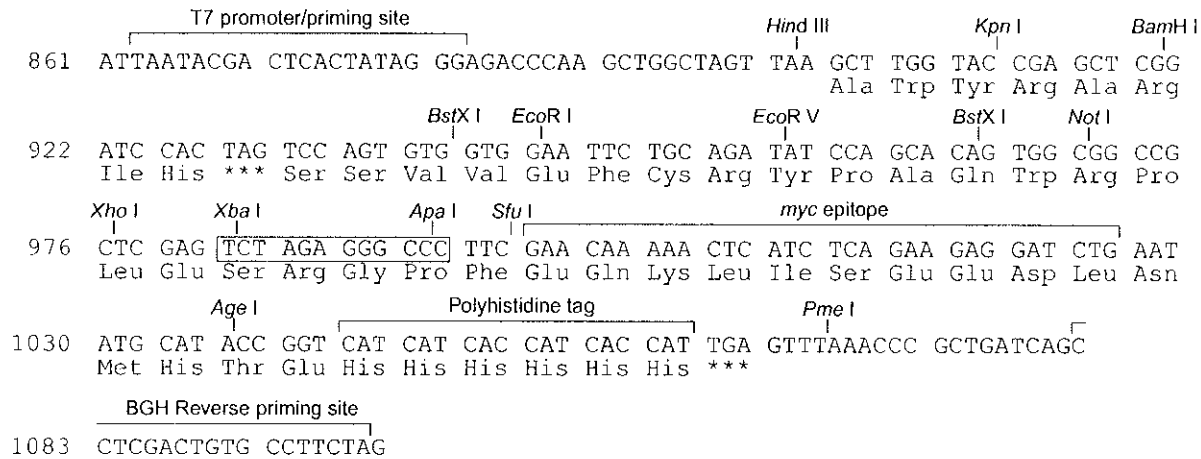
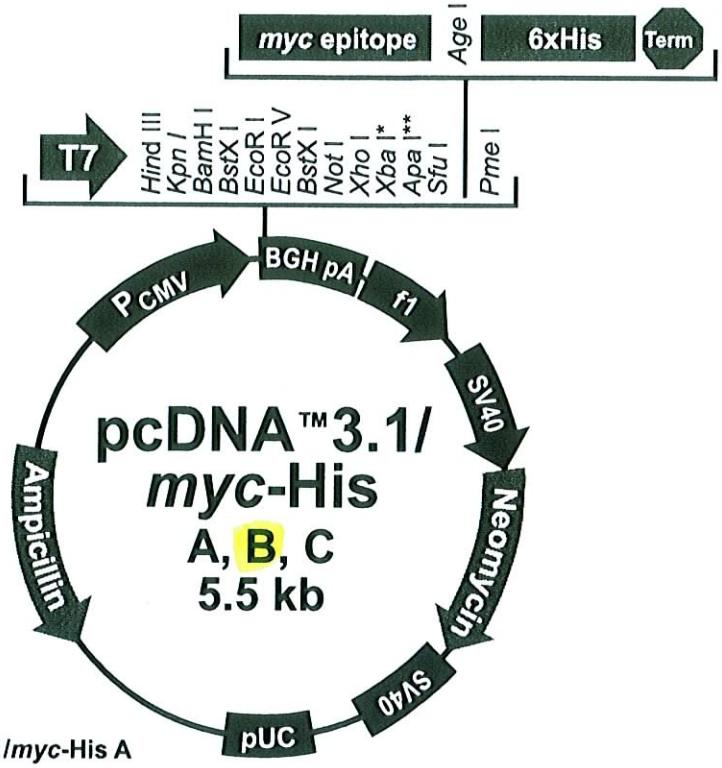
pcDNA™3.1/*myc*-His A MCS





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** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.

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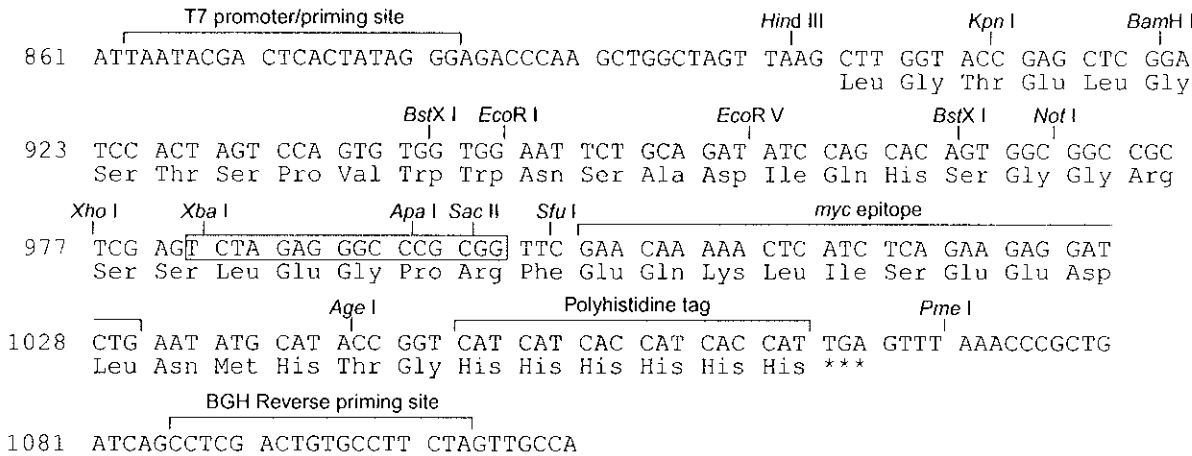
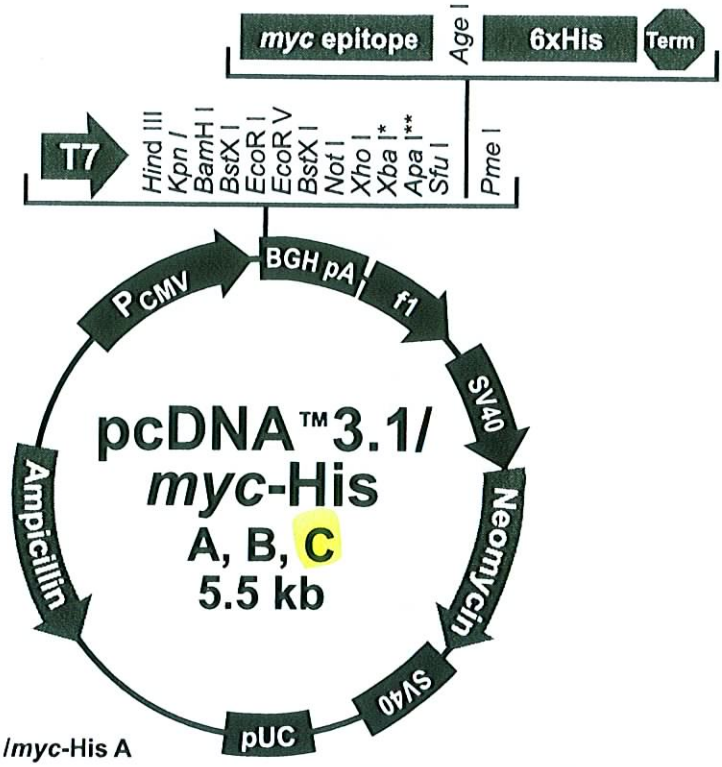
pcDNA™3.1/*myc*-His B MCS





Comments for pcDNA™3.1/*myc*-His A
5493 nucleotides

CMV promoter: bases 209-863
T7 promoter/priming site: bases 863-882
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* There is a unique *Bst*E II site, but no *Xba* I or *Apa* I sites in version C.

** There is a unique *Sac* II site between the *Apa* I site and the *Sfu* I site in version B only.

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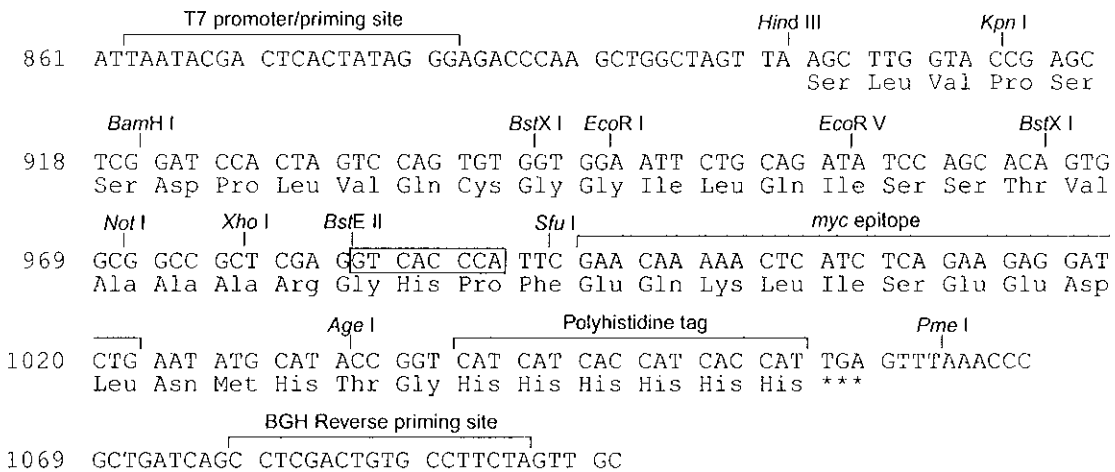
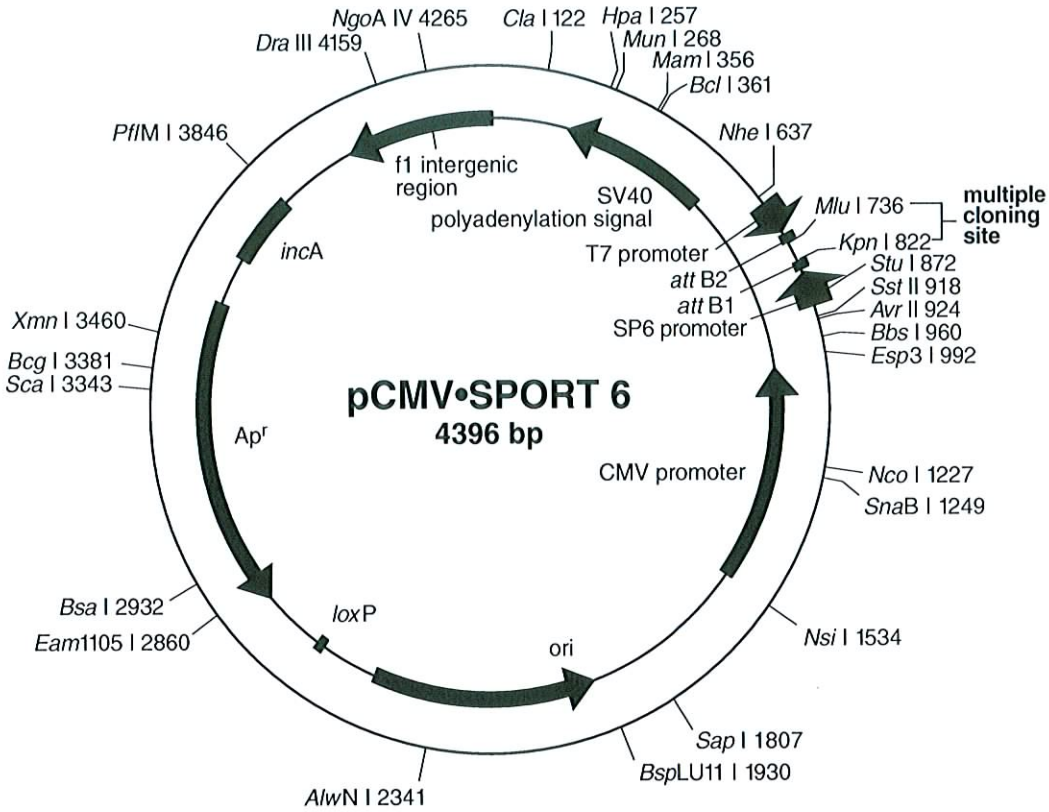
pcDNA™3.1/*myc*-His C MCS





pCMV•SPORT 6 multiple cloning site and primer binding regions: 641-917 (The sequence listed here is the (-) strand.)

M13/pUC Forward 23-Base Sequencing Primer
5 -CC CAGTCACGAC GTTGTAAAAC G-3 →

T7 Promoter Primer
5 -TAATACGACT CACTATAGGG-3 →

5 -GCAGTTTTCC CAGTCACGAC GTTGTAAAAC GACGGCCAGT GCCTAGCTTA TAATACGACT CACTATAGGG ACCACTTTGT ACAAGAAAGC TGGGTACGCG TAAGCTTGGG CCCCTCGAGG GATACTCTAG AGCGGCCGCCC

T7 promoter | att B2 | Mlu I | Hind III | Apa I | Xho I | Xba I | Not I

cDNA Insert: CGGACGCG TGGGTCGACG ATATCCCGGG AATTCCGGAC CGGTACCAGC CTGCTTTTTT GTACAAACTT GTTCTATAGT GTCACCTAAA TAGGCCTAAT GGTCATAGCT GTTTCCTGTG TGAAATTGTT ATCCGCT-3

Mlu I* | 800 | PinA I | SP6 promoter

Sal I | EcoR V | Sma I | EcoR I | Rsr II | Kpn I

← 3 -GATATCA CAGTGGATTT A-5 (SP6 Promoter Primer)

← 3 -GGACAC ACTTTAACAA TAGGCGA-5 (M13/pUC Reverse 23-Base Sequencing Primer)

att B1

* This *Mlu* I restriction site is contained within the *Sal* I adapter introduced into the vector upon ligation of the cDNA insert.

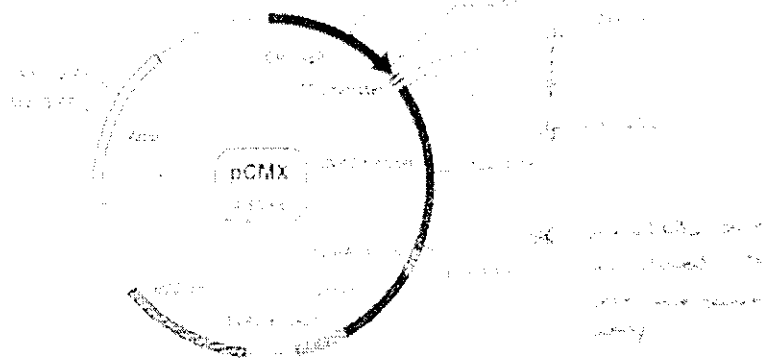
Figure 5. Map and Multiple Cloning Site of Plasmid pCMV•SPORT 6.

Invitrogen # 12209-011

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pCMX
Plasmid Type	Mammalian
Viral/Non-viral	non-viral
Constitutive/Inducible	constitutive
Promoter	CMV
Plasmid Size	4500
Bacterial Resistance	Ampicillin
Notes	Please see K. Umesono et al., (1991) Cell 65: 1255-1266. The map shown is from Inder Verma's lab. The wild-type version of pCMX plasmid does not contain IkB.

Maps



traction and a negative 36° twist around the double helix. How these changes promote or preclude binding and how the geometry of the bound receptor influences activity is unclear.

While the 3–4–5 rule provides an explanation for hormonal response of the VDR, TR, and RAR, it also leads to speculation that the remaining spacing options will serve as open slots for other members of the nuclear receptor family. The molecular underpinnings of this model are not yet known and will require a greater analysis of the interaction of the receptors with their cognate response elements to understand better its ultimate potential and limitations.

Experimental Procedures

Plasmids

Receptor expression plasmids used in the cotransfection assay were described previously (pRShTRβ, Thompson and Evans, 1989; pRShRARα, Giguére et al., 1987; pRShRARβ and pRShRARγ, Ishikawa et al., 1990; pRShVDR, Schüle et al., 1990). A basal reporter plasmid, ΔSV-CAT, was constructed by replacing the TK promoter in TK-CAT (Damm et al., 1989) with the SphI–HindIII fragment of the SV40 early promoter. All of the recombinant CAT reporter plasmids used in this study harbor a single copy of the indicated oligonucleotides at the unique HindIII site upstream of the SV40 promoter. Identity of the inserted oligonucleotides was confirmed by sequencing. To improve production of receptor proteins in COS cells, a new eukaryotic expression vector, pCMX, was prepared by modifying the plasmid CDM8 (Seed, 1987). The CDM8 was cut with MluI and StuI in order to release the DNA fragment encoding the CMV/T7 promoter, SV40 small t intron/poly(A) signal, polyoma virus enhancer/origin, and SV40 enhancer/origin. The resulting fragment was ligated to a larger fragment of PvuII-digested pUC19. An internal deletion was introduced between unique BamHI and BclI sites present in the CDM8 portion. The stuffer sequence flanked by XbaI sites was replaced with a synthetic polylinker coding for 5′-KpnI/Asp718–EcoRV–BamHI–MscI–NheI-3′, followed by a stretch of 5′-TAGGTAGCTAG-3′, which can function as a universal termination signal for protein translation. The coding sequence of the luciferase (de Wet et al., 1987) and human TRβ, RARα, and VDR was respectively placed in the polylinker region of the pCMX, generating pCMX-LUC, pCMX-hTRβ, pCMX-hRARα, and pCMX-hVDR. The translation start site of the RARα was modified to ACCACCATG by attaching the synthetic linker encoding a consensus translation start signal (Hollenberg and Evans, 1988). This modification resulted in a much better yield of the receptor translation as judged in the in vitro reticulocyte lysate translation system.

Cotransfection Assay

A monkey kidney cell line, CV-1, was kept in DMEM supplemented with 10% charcoal-resin double-split calf bovine serum. Transfections were performed via the calcium–phosphate precipitation method as described (Umesono and Evans, 1989) with 0.5 μg of a pRS receptor expression plasmid, 1.0 μg of a reporter CAT plasmid, 5 μg of pRAS-βGAL (Umesono and Evans, 1989) as an internal control, and 8.5 μg of carrier plasmid pUC19. The cells were transfected for 8 hr, and after the DNA precipitates were washed, they were incubated for an additional 36 hr with or without the ligand (T_3, 100 nM; RA, 1 μM; 1,25-$(OH)_2$ vitamin D_3, 100 nM). Cell extracts were prepared for βGAL and CAT assays as described (Umesono and Evans, 1989). Transfection of F9 teratocarcinoma cells was carried out with a similar method except the cells were incubated for 12 hr in the presence of the DNA precipitates and the RA was added at 1 μM for another 24 hr before harvesting the cells. Two, 5, and 4 μg of the reporter, pRAS-βGAL, and pUC19 were used, respectively.

DNA-Binding Assay

COS cells were cultured in DMEM with 10% calf bovine serum and transfected by the calcium–phosphate method with 20 μg of the pCMX receptor expression plasmid for 6 hr followed by a glycerol shock. After incubating the transfected COS cells for another 48 hr, the cells were harvested to prepare extracts for the DNA-binding assay as described by Damm et al. (1989). The extracts were made in 20 mM HEPES (pH 7.4), 0.4 M KCl, 2 mM DTT, and 20% glycerol. A similar method was employed to prepare a whole-cell extract from F9 stem cells. For the binding, 5 (COS) or 10 (F9) μg of protein was incubated first in 20 mM HEPES, 80 mM KCl, 1 mM DTT, 0.1% NP-40, 2.5 μg of poly(dI-dC), and 10% glycerol on ice for 20 min. Cold competitor oligonucleotides, when included, were added during this preincubation period. Then 40 fmol of ^{32}P-labeled oligonucleotide (1–2 × 10^5 cpm, prepared through filling-in reaction by Klenow polymerase in the presence of [α-^{32}P] dCTP) probe was added to the reaction followed by incubation at room temperature for 30 min. The receptor–DNA complexes were resolved by electrophoresis through a 5% polyacrylamide gel containing 5% glycerol at 6 V/cm at room temperature. Under the condition employed, inclusion of the ligand did not alter the DNA-binding pattern of the receptor proteins.

Acknowledgments

We thank Dr. Vijak Mahdavi for kindly providing us with pαTRE.A10-CAT and Dr. J. Wesley Pike for communicating their results prior to publication. We also thank Ester Banayo for her expert technical assistance, Drs. Mike McKeown, Inder Verma, Henry Sucov, David Mangelsdorf, Tony Oro, and Steve Kliewer for discussion and critical reading of the manuscript, and Elaine Stevens for administrative assistance and help in the preparation of the manuscript. K. U. is a Research Associate and R. M. E. is an Investigator of Howard Hughes Medical Institute at the Salk Institute for Biological Studies. This work was supported by the Howard Hughes Medical Institute, the National Institutes of Health, and the Mathers Foundation.

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Received March 20, 1991.

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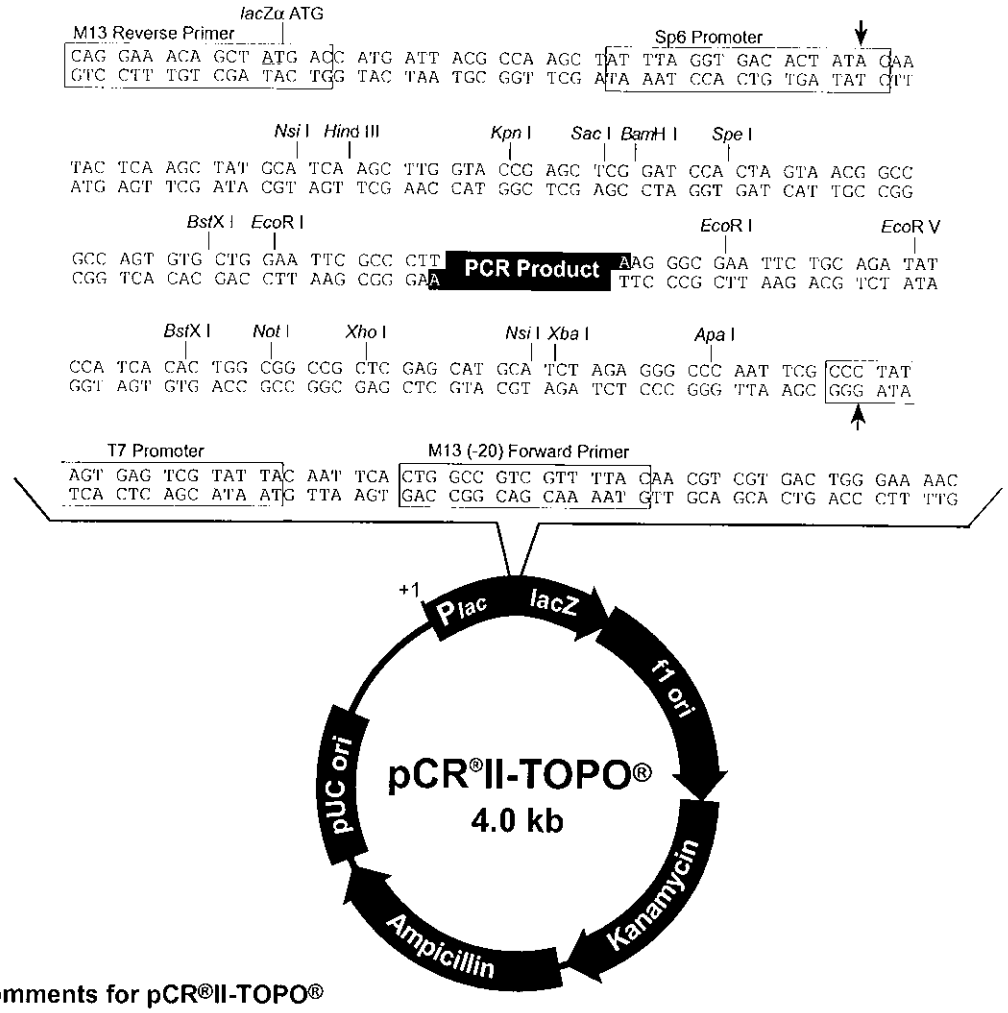
Burnside, J., Darling, D. S., and Chin, W. W. (1990). A nuclear factor that enhances binding of thyroid hormone receptors to thyroid response elements. J. Biol. Chem. *265*, 2500–2504.



Comments for pCR®II-TOPO®
3973 nucleotides

LacZα gene: bases 1-589
M13 Reverse priming site: bases 205-221
Sp6 promoter: bases 239-256
Multiple Cloning Site: bases 269-383
T7 promoter: bases 406-425
M13 (-20) Forward priming site: bases 433-448
f1 origin: bases 590-1027
Kanamycin resistance ORF: bases 1361-2155
Ampicillin resistance ORF: bases 2173-3033
pUC origin: bases 3178-3851

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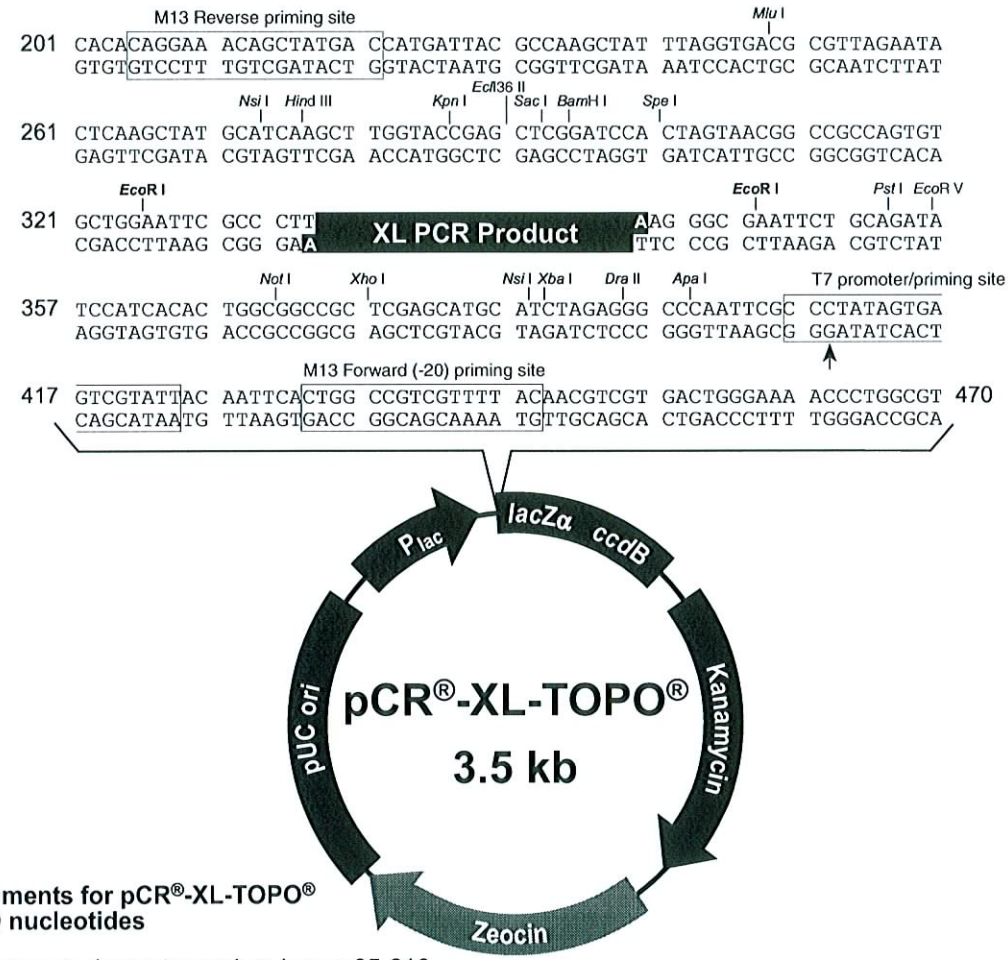
K4650-01

Map of pCR®-XL-TOPO®

pCR®-XL-TOPO® Map

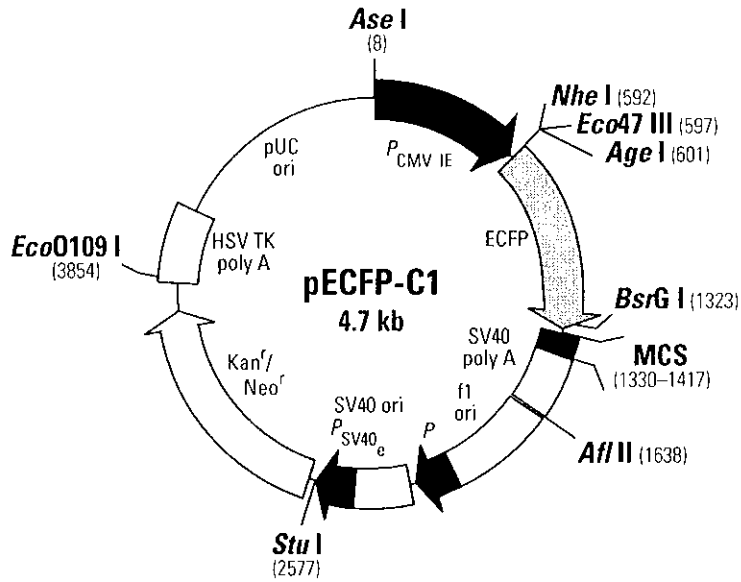
The map below shows the features of pCR®-XL-TOPO® and the sequence surrounding the TOPO TA Cloning® site. Restriction sites are labeled to indicate the actual cleavage site. The arrow indicates the start of transcription for T7 polymerase. The complete sequence of the vector is available for downloading from our **Web site (www.invitrogen.com) or by contacting Technical Service (page 19)**.

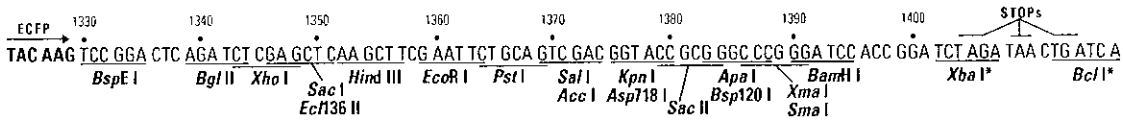


Comments for pCR®-XL-TOPO®
3519 nucleotides

Lac promoter/operator region: bases 95-216
M13 Reverse priming site: bases 205-221
Lac Za ORF: bases 217-576
Multiple Cloning Site: bases 248-399
TOPO® Cloning site: bases 336-337
T7 promoter priming site: bases 406-425
M13 Forward (-20) priming site: bases 433-448
Fusion joint: bases 577-585
*ccd*B lethal gene ORF: bases 586-888
Kanamycin resistance ORF: bases 1237-2031
Zeocin resistance ORF: bases 2238-2612
pUC origin: bases 2680-3393

Invitrogen K4750-10





Restriction map and multiple cloning site (MCS) of pECFP-C1. Unique restriction sites are in bold. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pECFP-C1 encodes an enhanced cyan fluorescent variant of the *Aequorea victoria* green fluorescent protein gene (GFP). The ECFP gene contains six amino acid substitutions. The Tyr-66 to Trp substitution gives ECFP fluorescence excitation (major peak at 433 nm and a minor peak at 453 nm) and emission (major peak at 475 nm and a minor peak at 501 nm) similar to other cyan emission variants (1–3). The other five substitutions (Phe-64 to Leu; Ser-65 to Thr; Asn-146 to Ile; Met-153 to Thr; and Val-163 to Ala) enhance the brightness and solubility of the protein, primarily due to improved protein-folding properties and efficiency of chromophore formation (2, 4, 5).

In addition to the chromophore mutations, ECFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (6). Furthermore, upstream sequences flanking ECFP have been converted to a Kozak consensus translation initiation site (7). These changes increase the translational efficiency of the ECFP mRNA and consequently the expression of ECFP in mammalian and plant cells.

The MCS in pECFP-C1 is between the ECFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of ECFP if they are in the same reading frame as ECFP and there are no intervening in-frame stop codons. ECFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neor) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neor expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

(PR29964; published 03 October 2002)

The recombinant ECFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (8). pECFP-C1 can also be used simply to express ECFP 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
- Enhanced cyan fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  ECFP mutations (Phe-64 to Leu; Ser-65 to Thr; and Tyr-66 to Trp): 805–813; Asn-146 to Ile: 1051-1053; Met-153 to Thr: 1072–1074; Val-163 to Ala: 1102-1104
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in ECFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of ECFP.)
- Bacterial promoter for expression of Kan^r gene.
  –35 region: 2164–2169; –10 region: 2187–2192
  Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2627–2629; stop codon: 3419–3421
  G→A mutation to remove *Pst* I site: 2809
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 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:

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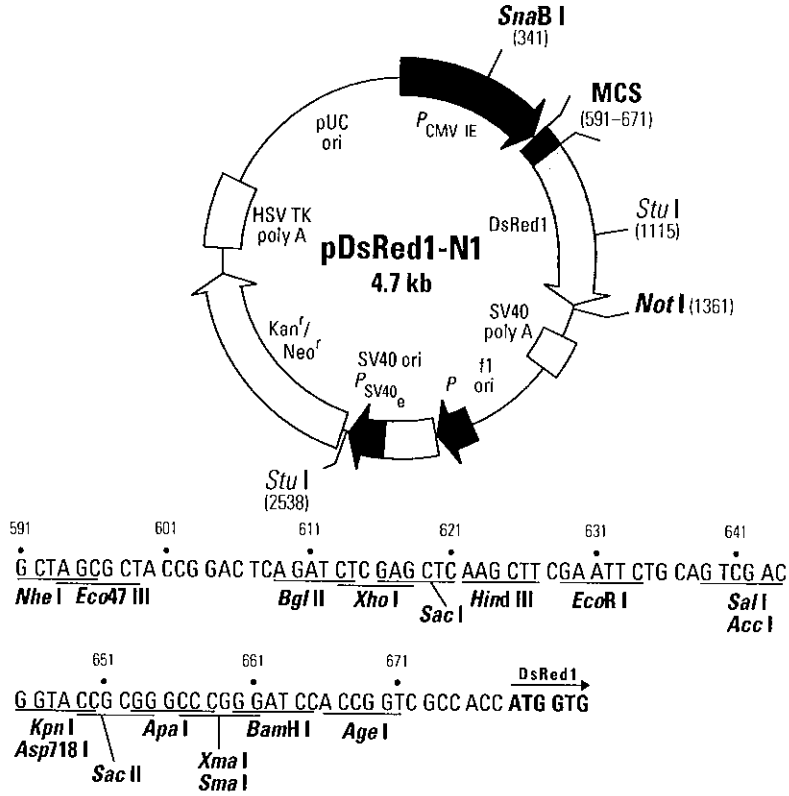
6. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
7. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
8. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II*, Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

pDsRed1-N1 Vector Information PT3405-5

GenBank Accession #: Submission in progress Catalog #6921-1



Restriction Map and Multiple Cloning Site (MCS) of pDsRed1-N1 Vector. Unique restriction sites are in bold. The *Not* I site follows the DsRed1 stop codon.

Description

pDsRed1-N1 encodes a novel red fluorescent protein (RFP; 1) that has been optimized for high expression in mammalian cells (excitation maximum = 558 nm; emission maximum = 583 nm). RFP was isolated from an IndoPacific sea anemone-relative, *Discosoma sp*; DsRed1's coding sequence contains 144 silent base pair changes, which correspond to human codon-usage preferences for high expression in mammalian cells (2). Sequences upstream of DsRed1 have been converted to a Kozak consensus translation initiation site (3) to increase translation efficiency in eukaryotic cells. The MCS is between the immediate early promoter of CMV ($P_{CMV\ IE}$) and the DsRed1 coding sequence. Genes cloned into the MCS as described below are expressed as fusions to the N-terminus of DsRed1. SV40 polyadenylation signals downstream of the DsRed1 gene direct proper processing of the 3' end of the DsRed1 mRNA. The vector backbone contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (Neor) allows stably transfected eukaryotic cells to be selected using G418. This cassette consists 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. A bacterial promoter upstream of the cassette confers kanamycin resistance to *E. coli*. The pDsRed1-N1 backbone also has a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

Fusions to the N terminus of DsRed1 typically do not alter the fluorescence properties of native DsRed1, allowing *in vivo* localization of the fusion protein.The target gene should be cloned into pDsRed1-N1 in frame with the DsRed1 coding sequence, with no intervening in-frame stop codons. The inserted gene should include an initiating ATG codon. Recombinant pDsRed1-N1 can be transfected into mammalian cells using any standard transfection method. If required, stable transfectants can be selected using G418 (4). Unmodified pDsRed1-N1 can also be used to express DsRed1 in a cell line of interest (*e.g.*, for use as a transfection marker).

(PR07925; published 14 July 2000)

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
- *Discosoma sp.* Red Fluorescent Protein (DsRed1) gene
  Kozak consensus translation initiation site: 672–682
  Start codon (ATG): 679–681; Stop codon: 1357–1359
  Insertion of Val at position 2: 682–684
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1511–1516 & 1540–1545; mRNA 3' ends: 1549 & 1561
- f1 single-strand DNA origin: 1608–2063 (Packages the noncoding strand of DsRed1.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2125–2130; –10 region: 2148–2153
  Transcription start point: 2160
- SV40 origin of replication: 2404–2539
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2237–2308 & 2309–2380
  21-bp repeats: 2384–2404, 2405–2425 & 2427–2447
  Early promoter element: 2460–2466
  Major transcription start points: 2456, 2494, 2500 & 2505
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences: start codon (ATG): 2588–2590; stop codon: 3380–3382
  G→A mutation to remove *Pst* I site: 2770
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3116
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3618–3623 & 3631–3636
- pUC plasmid replication origin: 3967–4610

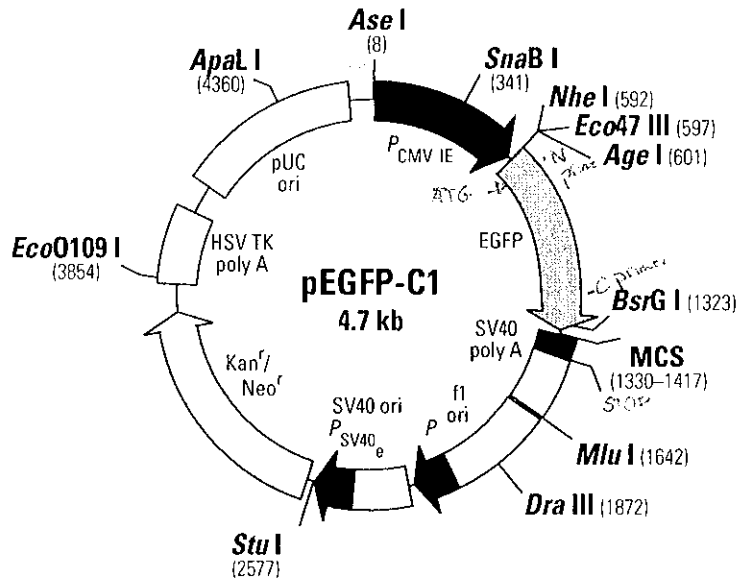
Propagation in *E. coli*

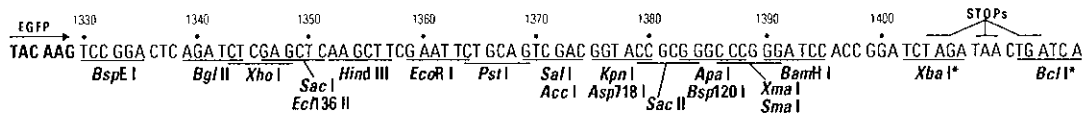
- Suitable host strains: DH5α, HB101 and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 or XL1-Blue.
- Selectable marker: plasmid confers resistance to kanamycin (30 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ≈500
- Plasmid incompatibility group: pMB1/ColE1

References

1. Matz, M. V., *et al.* (1999) *Nature Biotech.* **17**:969–973.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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 CLONTECH. This vector has not been completely sequenced.





Restriction Map and Multiple Cloning Site (MCS) of pEGFP-C1. All restriction sites shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vector with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description

pEGFP-C1 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-C1 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-C1 is between the EGFP coding sequences and the SV40 poly A. Genes cloned into the MCS will be expressed as fusions to the C-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 (Neor), 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-C1 backbone also provides a pUC origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Use

Fusions to the C 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-C1 so that it is in frame with the EGFP coding sequences, with no intervening in-frame stop codons. 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-C1 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
- Enhanced green fluorescent protein gene
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; Stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  GFPmut1 chromophore mutations (Phe-64 to Leu; Ser-65 to Thr): 805–810
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EGFP.)
- Bacterial promoter for expression of Kan^r gene
  –35 region: 2164–2169; –10 region: 2187–2192
  Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464, & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2627–2629; stop codon: 3419–3421
  G→A mutation to remove *Pst* I site: 2809
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 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

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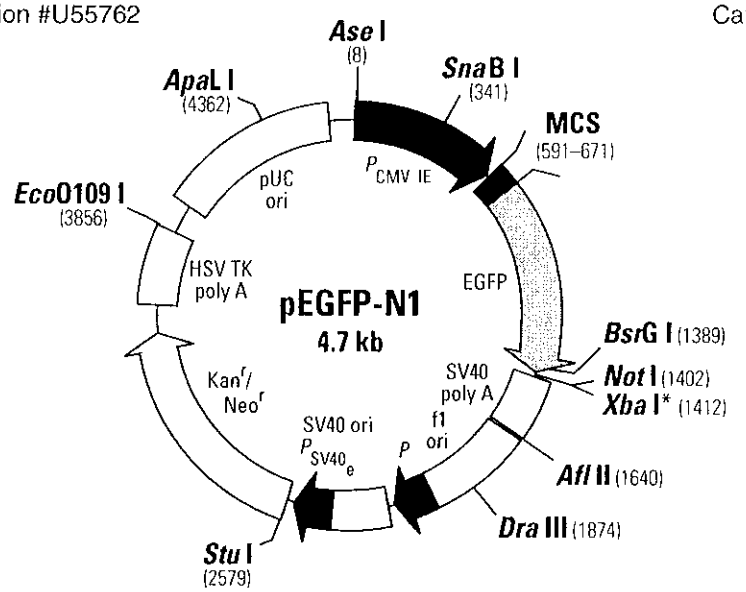
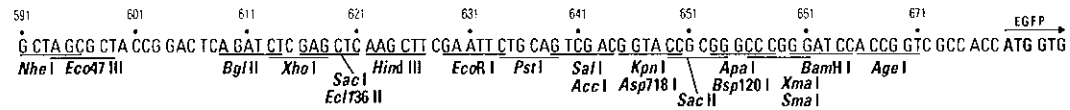
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
7.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

pEGFP-N1 Vector Information PT3027-5

GenBank Accession #U55762 Catalog #6085-1





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 (Neor), 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.

(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 promoterfor 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:

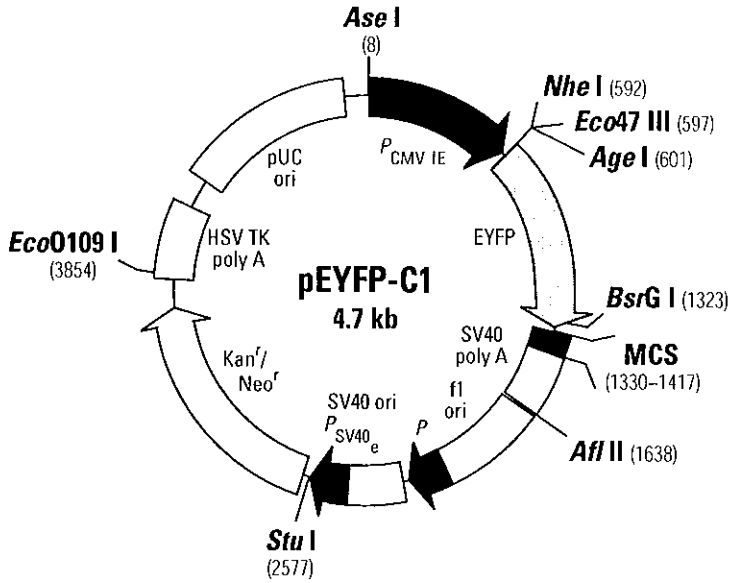
1. Prasher, D. C., *et al.* (1992) *Gene* **111**:229–233.
2. Chalfie, M., *et al.* (1994) *Science* **263**:802–805.
3. Inouye, S. & Tsuji, F. I. (1994) *FEBS Letters* **341**:277–280.
4. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
5. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
6. 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.

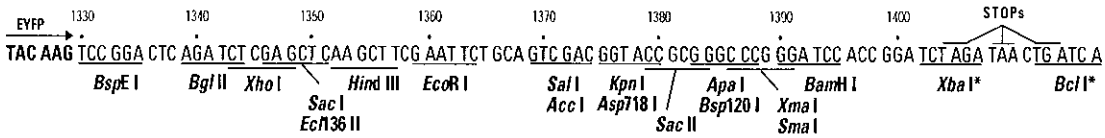
Vector Information

pEYFP-C1 Vector Information

PT3176-5

Catalog #6005-1





Restriction map and multiple cloning site (MCS) of pEYFP-C1. All restriction sites are shown are unique. The *Xba* I and *Bcl* I sites (*) are methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest the vectors with these enzymes, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-C1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains the four amino acid substitutions previously published as GFP-10C (1): Ser-65 to Gly; Val-68 to Leu; Ser-72 to Ala; and and Thr-203 to Tyr. The fluorescence excitation maximum of EYFP is 513 nm; the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513-nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescent quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence observed is roughly equivalent to that from EGFP.

A mixture of EYFP- and EGFP-expressing cells can be sorted by flow cytometry using a single excitation wavelength (i.e., 488 nm). EYFP emission is detected using a 525-nm dichroic shortpass mirror and a 530/30-nm bandpass filter; EGFP emission is detected using a 510/20-nm bandpass filter.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-C1 is between the EYFP coding sequence and the stop codon. Genes cloned into the MCS will be expressed as fusions to the C-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening in-frame stop codons. EYFP with a C-terminal fusion moiety retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin for replication and a neomycin resistance (Neor) gene for selection (using G418) in eukaryotic cells. A bacterial promoter (*P*) upstream of Neor expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production. The recombinant EYFP vector can be

(PR29944; published 03 October 2002)

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transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pEYFP-C1 can also be used simply to express EYFP 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
- Enhanced yellow fluorescent protein (EYFP) gene:
  Kozak consensus translation initiation site: 606–616
  Start codon (ATG): 613–615; stop codon: 1408–1410
  Insertion of Val at position 2: 616–618
  GFP-10C mutations (Ser-65 to Gly: 808–810; Val-68 to Leu: 817–819; Ser-72 to Ala: 829–831; Thr-203 to Tyr: 1222–1224)
  His-231 to Leu mutation (A→T): 1307
  Last amino acid in wild-type GFP coding region: 1327–1329
- MCS: 1330–1417
- SV40 early mRNA polyadenylation signal:
  Polyadenylation signals: 1550–1555 & 1579–1584; mRNA 3' ends: 1588 & 1600
- f1 single-strand DNA origin: 1647–2102 (Packages the noncoding strand of EYFP.)
- Bacterial promoter for expression of Kan^r gene:
  –35 region: 2164–2169; –10 region: 2187–2192
  Transcription start point: 2199
- SV40 origin of replication: 2443–2578
- SV40 early promoter:
  Enhancer (72-bp tandem repeats): 2276–2347 & 2348–2419
  21-bp repeats: 2423–2443, 2444–2464 & 2466–2486
  Early promoter element: 2499–2505
  Major transcription start points: 2495, 2533, 2539 & 2544
- Kanamycin/neomycin resistance gene:
  Neomycin phosphotransferase coding sequences:
  Start codon (ATG): 2627–2629; stop codon: 3419–3421
  G→A mutation to remove *Pst* I site: 2809
  C→A (Arg to Ser) mutation to remove *Bss*H II site: 3155
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal
  Polyadenylation signals: 3657–3662 & 3670–3675
- pUC plasmid replication origin: 4006–4649

Primer Locations:

- EGFP-N Sequencing Primer (#6479-1): 679–658
- EGFP-C Sequencing Primer (#6478-1): 1266–1287

Propagation in *E. coli*:

- Suitable host strains: DH5α, HB101, and other general purpose strains. Single-stranded DNA production requires a host containing an F plasmid such as JM109 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. Ormö, M., *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. Gorman, C. (1985) In *DNA Cloning: A Practical Approach, Vol. II,* Ed. Glover, D. M. (IRL Press, Oxford, UK), pp. 143–190.

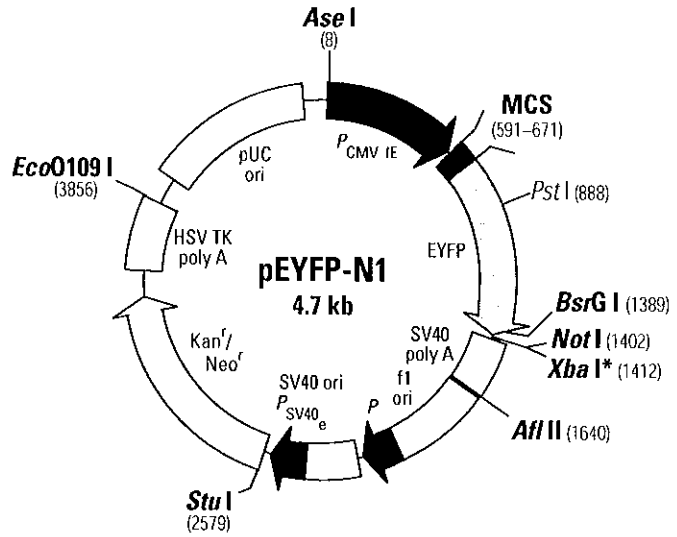
Note: The attached sequence file has been compiled from information in the sequence databases, published literature, and other sources, together with partial sequences obtained by BD Biosciences Clontech. This vector has not been completely sequenced.

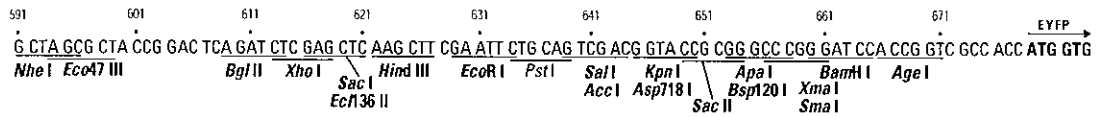
Vector Information

pEYFP-N1 Vector Information

PT3192-5

Catalog #6006-1





Restriction Map and Multiple Cloning Site (MCS) of pEYFP-N1. Unique restriction sites are in bold. The *Xba* I site (*) is methylated in the DNA provided by BD Biosciences Clontech. If you wish to digest this vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA.

Description:

pEYFP-N1 encodes an enhanced yellow-green variant of the *Aequorea victoria* green fluorescent protein (GFP). The EYFP gene contains four amino acid substitutions previously published as GFP-10C (1). The fluorescence excitation maximum of EYFP is 513 nm, and the emission spectrum has a peak at 527 nm (in the yellow-green region). When excited at 513 nm, the E_m of EYFP is 36,500 $cm^{-1}M^{-1}$ and the fluorescence quantum yield is 0.63 (1), resulting in a bright fluorescent signal. The fluorescence level observed from EYFP is roughly equivalent to that from EGFP.

In addition to the chromophore mutations, EYFP contains >190 silent mutations that create an open reading frame comprised almost entirely of preferred human codons (2). Furthermore, upstream sequences flanking EYFP have been converted to a Kozak consensus translation initiation site (3). These changes increase the translational efficiency of the EYFP mRNA and consequently the expression of EYFP in mammalian and plant cells.

The MCS in pEYFP-N1 is between the immediate early promoter of CMV ($P_{CMV\,IE}$) and the EYFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EYFP if they are in the same reading frame as EYFP and there are no intervening stop codons. The inserted gene should include an initiating ATG codon. EYFP with N-terminal fusion moieties retains the fluorescent properties of the native protein and thus can be used to localize fusion proteins *in vivo*.

The vector contains an SV40 origin of replication and a neomycin resistance (Neo^r) gene for selection (using G418) in mammalian cells. A bacterial promoter upstream of this cassette (*P*) expresses kanamycin resistance in *E. coli*. The vector backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

The recombinant EYFP vector can be transfected into mammalian cells using any standard transfection method. If required, stable transformants can be selected using G418 (4). pEYFP-N1 can also be used simply to express EYFP in a cell line of interest (e.g., as a transfection marker). EGFP, EYFP, and EBFP variants can be used independently or in combination for flow cytometry analysis.

(PR29945; published 03 Octobers 2002)

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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 yellow fluorescent protein (EYFP) 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
  GFP-10C mutations (Ser-65 to Gly: 874–876; Val-68 to Leu: 883–885; Ser-72 to Ala: 895–897; Thr-203 to Tyr: 1288–1290)
  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 EYFP.)
- 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: DH5α, 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) in *E. coli* hosts.
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/ColE1

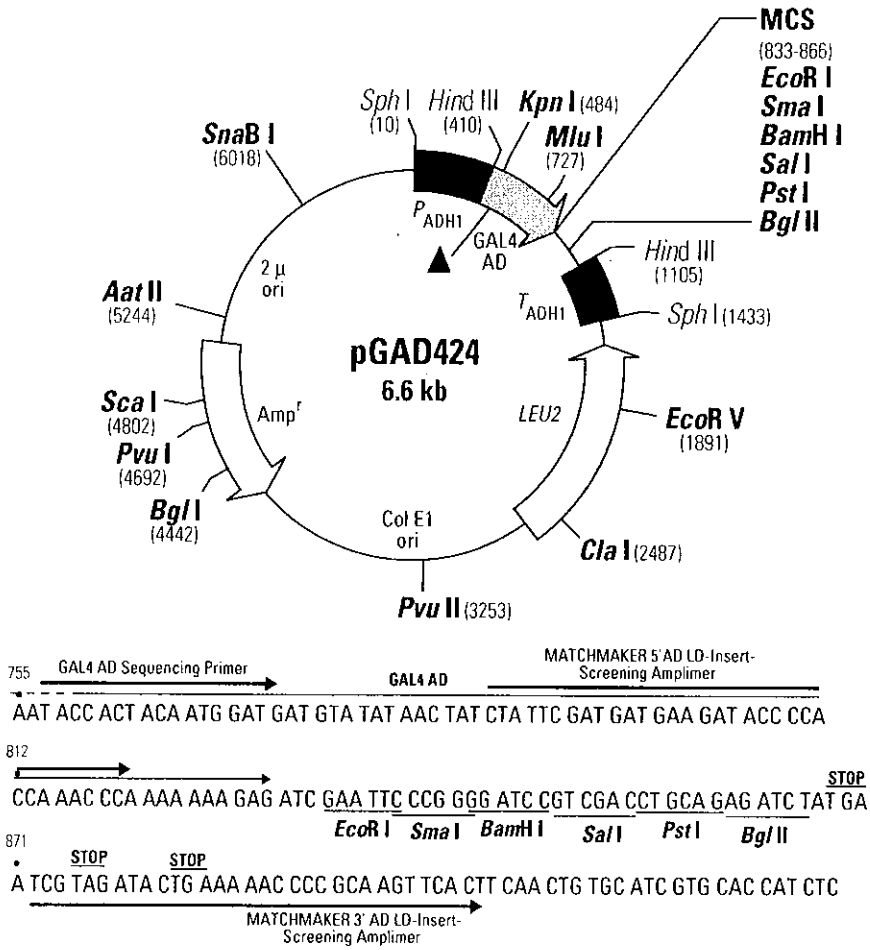
References:

1. Ormö, M. *et al.* (1996) *Science* **273**:1392–1395.
2. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
3. Kozak, M. (1987) *Nucleic Acids Res.* **15**:8125–8148.
4. 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.

pGAD424 AD Vector Information PT1022-5

GenBank Accession #: U07647 Catalog #K1605-1



Restriction map and multiple cloning site (MCS) of pGAD424. Unique restriction sites are in bold.

Description:

pGAD424 generates a hybrid protein that contains the sequences for the GAL4 activation domain (AD; a.a. 768–881) (1). pGAD424 has unique restriction sites located in the MCS region at the 3'-end of the open reading frame for the GAL4 AD sequence. For the construction of a hybrid protein, the gene encoding the protein of interest (or a collection of cDNAs) is ligated into the MCS in the correct orientation and in the correct reading frame such that a fusion protein is generated. The fusion protein is expressed at high levels in yeast host cells from the constitutive *ADH1* promoter; transcription is terminated at the *ADH1* transcription termination signal. The hybrid protein is targeted to the yeast nucleus by nuclear localization sequences that have been added to the AD sequence from a heterologous source (2). pGAD424 is a shuttle vector that replicates autonomously in both *E. coli* and *S. cerevisiae*. It carries the *bla* gene (for ampicillin resistance in *E. coli*) and the *LEU2* nutritional marker that allow yeast auxotrophs carrying pGAD424 to grow on limiting synthetic medium lacking Leu.

Location of features

- Promoter fragment carrying the truncated *S. cerevisiae ADH1* promoter: 10–406
- GAL4 activation domain (AD) polypeptide
  start codon (ATG): 422–424; stop codon: 875–877; GAL4 codons 768–881: 491–829
  SV40 T-antigen nuclear localization signal: 452–472
- Multiple cloning site: 834–866
- Translation stop codons: 868–870, 875–877 & 882–884
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator: 1105–1432
- *LEU2* coding sequences: start codon (ATG): 2640–2638; stop codon: 1548–1546
- Col E1 plasmid replication origin: 3457–4100
- Ampicillin resistance gene
  Promoter: –35 region: 5178–5173; –10 region: 5155–5150
  Transcription start point: 5143
  Ribosome binding site: 5120–5116
  β-lactamase coding sequences:
  Start codon (ATG): 5108–5106; stop codon: 4250–4248
  β-lactamase signal peptide: 5108–5040
  β-lactamase mature protein: 5039–4251

Primer locations

- MATCHMAKER 5' AD LD-Insert Screening Amplimer (#9103-1): 757–773
- MATCHMAKER 3' AD LD-Insert Screening Amplimer (#9103-1): 908–889

Propagation in *E. coli*

- Suitable host strains: DH5a, DH10 & other general purpose strains.
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts.
- *E. coli* replication origin: Col E1
- Copy number: 15–20

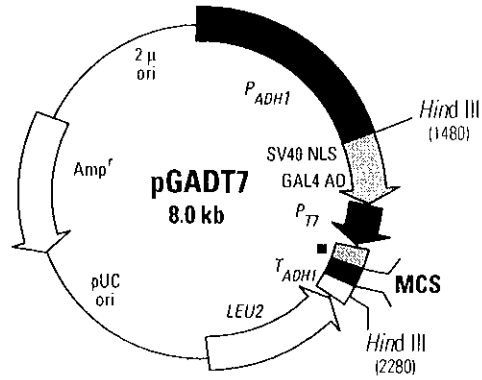
Propagation in *S. cerevisiae*

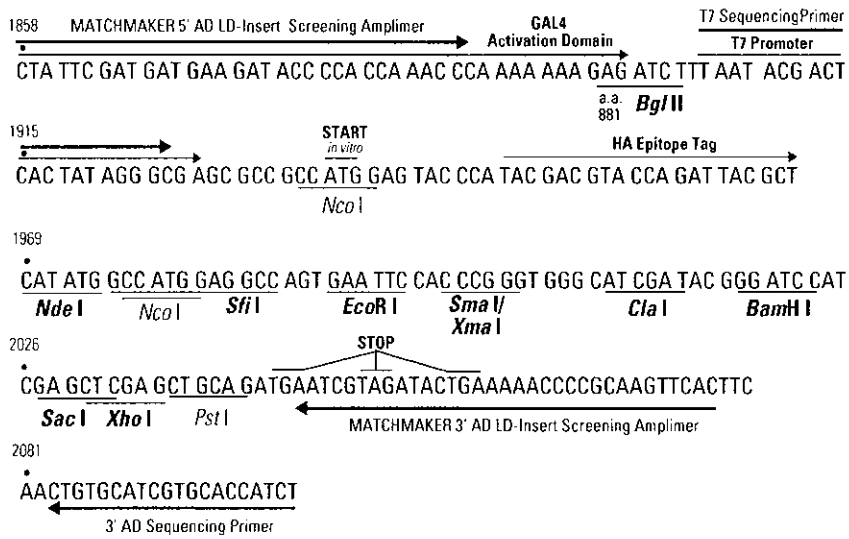
- Suitable host strains: Y187(α), Y190(a), SFY526(a), CG1945(a), or HF7c(a)
- Selectable marker: *LEU2*
- *S. cerevisiae* origin: 2 μ

References

1. Bartel, P. L., *et al.* (1993) In *Cellular Interactions in Development: A Practical Approach* (Oxford University Press, Oxford) pp. 135–179.
2. Chien, C. T., *et al.* (1991) *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.

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 CLONTECH. This vector has not been completely sequenced.





Restriction Map and Multiple Cloning Site (MCS) of pGADT7. Unique restriction sites are in bold.

Description:

The pGADT7 vector expresses proteins fused to amino acids 768–881 of the GAL4 activation domain (AD). In yeast, fusion proteins are expressed at high levels from the constitutive *ADH1* promoter (P_{ADH1}); transcription is terminated at the *ADH1* transcription termination signal (T_{ADH1}). The fusion protein is targeted to the yeast nucleus by the SV40 nuclear localization sequences that have been added to the activation domain sequence (1). pGADT7 also contains the T7 promoter, an HA epitope tag, and a MCS. pGADT7 replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 µ ori, respectively. The vector carries Amp^r for selection in *E. coli* and the *LEU2* nutritional marker for selection in yeast.

Use:

pGADT7 is the AD Vector included with MATCHMAKER Two-Hybrid System 3. The MCS of pGADT7 has unique restriction sites in frame with the 3'-end of the GAL4 AD for constructing a fusion protein with either a protein of interest or a fusion protein library. The bait protein is also expressed

as a fusion to a hemagglutinin (HA) epitope tag. HA-tagged proteins can be identified with antibodies raised to this common epitope, eliminating the need to generate specific antibodies to new proteins. The T7 promoter is used for *in vitro* transcription and translation of the epitope tagged fusion protein and also provides a binding site for sequencing using the T7 Sequencing Primer. Note that the AD is not expressed during the *in vitro* transcription and translation reactions.

The *Nco* I and *Pst* I sites may be used to shuttle inserts from pGADT7 into pGBKT7, the MATCHMAKER Two-Hybrid System 3 DNA-BD Vector. The MCS in pGADT7 is compatible with those in pMyc-CMV and pHA-CMV, CLONTECH's epitope tagged mammalian expression vector set (#K6003-1). As a result, the target gene can be shuttled into these vectors in order to confirm protein interactions *in vivo*.

Location of features:

- Full-length *S. cerevisiae ADH1* promoter (P_{ADH1}): 7–1479
- GAL4 AD polypeptide with SV40 Nuclear Localization Signal (NLS)
  NLS: 1501–1557
  GAL4 amino acids 768–881: 1561–1899
- T7 RNA polymerase promoter: 1905–1927
- HA epitope tag: 1942–1968
- Multiple Cloning Sites: 1969–2041
- Transcription termination signal
  Fragment carrying the *S. cerevisiae ADH1* terminator (T_{ADH1}): 2280–2605
- *LEU2* coding sequences: 3814–2723
- pUC plasmid replication origin: 4581–5418
- Ampicillin resistance gene: 6432–5575
- Yeast 2 μ replication origin: 6998–7988

Location of primers:

- T7 Sequencing Primer: 1905–1925
- 3' AD Sequencing Primer: 2102–2083
- MATCHMAKER 5' AD LD-Insert Screening Amplimer (#9103-1): 1858–1889
- MATCHMAKER 3' AD LD-Insert Screening Amplimer (#9103-1): 2078–2046

Propagation in *E. coli*:

- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 μg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Propagation in *S. cerevisiae*:

- Suitable host strains: Y187(α), Y190(a), SFY526(a), CG1945(a), HF7c(a), or AH109(a)
- Selectable marker: *LEU2*
- *S. cerevisiae* origin: 2 μ

Reference:

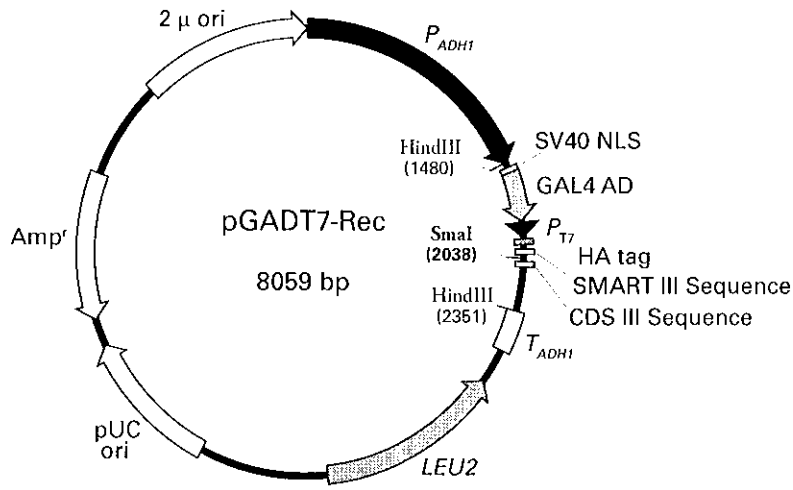
1. Chien, C. T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.

Vector Information

pGADT7-Rec Vector Information

PT3530-5

Sold as part of Cat. Nos. 630490 & 630491



SMART™ III terminus

MATCHMAKER 5' AD LD-Insert Screening Amplimer — GAL4 Activation Domain (a.a. 881) — T7 Sequencing Primer — T7 Promoter

1858 CTA TTC GAT GAT GAA GAT ACC CCA CCA AAC CCA AAA AAA GAG ATC TTT AAT ACG ACT

START *in vitro* — HA Epitope Tag

1915 CAC TAT AGG GCG AGC GCC GCC ATG GAG TAC CCA TAC GAC GTA CCA GAT TAC GCT

SMART III Oligonucleotide Sequence

1969 CAT ATG GCC ATG GAG GCC AGT GAA TTC CAC CCA AGC AGT GGT ATC AAC GCA GAG TGG

2026 CCA TTA TGG CCC

Partial SmaI site

CDS III terminus

CDS III Primer Sequence

2038 GGG AAA AAA CAT GTC GGC CGC CTC GGC CTC TAG AGG GTG GGC ATC GAT ACG GGA TCC

Partial SmaI site

STOP

2095 ATC GAG CTC GAG CTG CAG ATG AAT CGT AGA TAC TGA AAAACCCCGCAAGTTCACTTC

MATCHMAKER 3' AD LD-Insert Screening Amplimer

2152 AACTGTGCATCGTGCACCATCT

3' AD Sequencing Primer

Figure 1. pGADT7-Rec Vector Map and Cloning Site. A unique restriction site (SmaI) is shown in bold. Both the Make Your Own "Mate & Plate" Library System and the Matchmaker™ Gold Yeast One-Hybrid System (Cat. Nos. 630490 and 630491, respectively) contain the SmaI-linearized form of this vector, the form used for recombination-mediated cloning in yeast.

(PR8Z2660 published 24 December 2008)

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Description

In yeast, pGADT7-Rec expresses a protein of interest as a GAL4 activation domain (GAL4 AD) fusion. Transcription starts with the constitutive *ADH1* promoter (P_{ADH1}) and ends with the *ADH1* termination signal (T_{ADH1}). The GAL4 AD sequence includes the SV40 nuclear localization signal (SV40 NLS; 1) so that fusions translocate to the yeast nucleus. GAL4 AD fusions also contain a hemagglutinin epitope tag (HA tag) for easy identification with Clontech's HA-Tag Polyclonal Antibody (Cat. No. 631207).

The T7 promoter in pGADT7-Rec allows *in vitro* transcription and translation of the hemagglutinin (HA)-tagged fusion protein. It also provides a binding site for the T7 Sequencing Primer. In its circular form, pGADT7-Rec replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 μ ori, respectively. The vector carries Amp^r for selection in *E. coli* and the *LEU2* nutritional marker for selection in yeast.

Use

pGADT7-Rec is engineered for the construction of GAL4 AD/cDNA libraries by homologous recombination in yeast (Figure 2). Libraries made with this vector can be used for Matchmaker™ Gold One- and Two-Hybrid Screening.

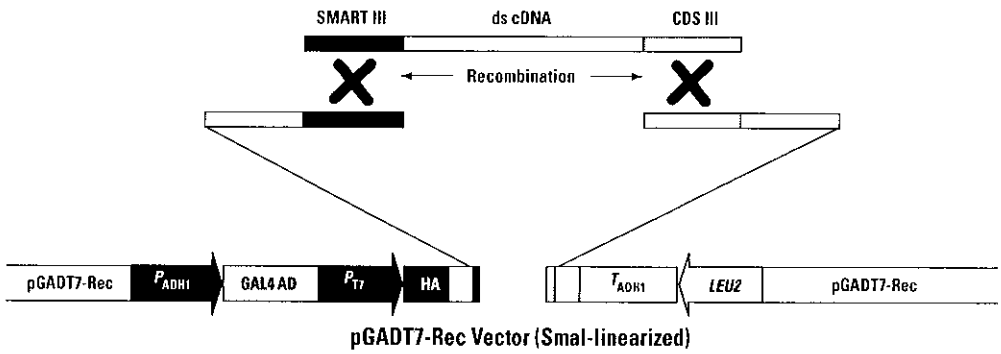


Figure 2. Cloning cDNA into pGADT7-Rec by homologous recombination *in vivo*. The ends of the SmaI-linearized vector are homologous to Clontech's SMART™ III Oligonucleotide and CDS III Primer, used in the Matchmaker cDNA synthesis protocol (Figure 1).

Location of features

- P_{ADH1} (full-length *S. cerevisiae ADH1* promoter): 7–1479
- GAL4 AD (GAL4 activation domain with SV40 nuclear localization signal [NLS]):
  SV40 NLS: 1501–1557
  GAL4 AD (amino acids 768–881): 1561–1899
- P_{T7} (T7 RNA polymerase promoter): 1905–1927
- HA tag (hemagglutinin epitope tag): 1942–1968
- SMART III Oligonucleotide sequence: 2001–2036
- CDS III Primer sequence: 2047–2071
- T_{ADH1} (*S. cerevisiae ADH1* Terminator): 2351–2676
- *LEU2* coding sequences: 2794–3885 (complementary)
- pUC ori (pUC replication origin): 4652–5489
- Amp^r (ampicillin resistance gene): 5646–6503 (complementary)
- 2 μ ori (yeast 2 μ replication origin): 7069–8059

Location of primers

- T7 Sequencing Primer: 1905–1927
- 3' AD Sequencing Primer: 2173–2154
- Matchmaker 5' AD LD-Insert Screening Amplimer (Cat. No. 630433): 1858–1889
- Matchmaker 3' AD LD-Insert Screening Amplimer (Cat. No. 630433): 2149–2117

Propagation in *E. coli*

- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to ampicillin (100 µg/ml) to *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Propagation in *S. cerevisiae*

- Suitable host strains: Y1HGold, Y2HGold, AH109(*MAT***a**), Y187(*MAT*α), Y190(*MAT***a**), SFY526(*MAT***a**), CG1945(*MAT***a**), HF7c(*MAT***a**)
- Selectable marker: *LEU2*
- *S. cerevisiae* origin: 2 µ

Reference

1. Chien, C.T., Bartel, P. L., Sternglanz, R. & Fields, S. (1991) *Proc. Natl. Acad. Sci. USA* **88**:9578–9582.

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

Appendix D: Two-Hybrid Control Vector Information

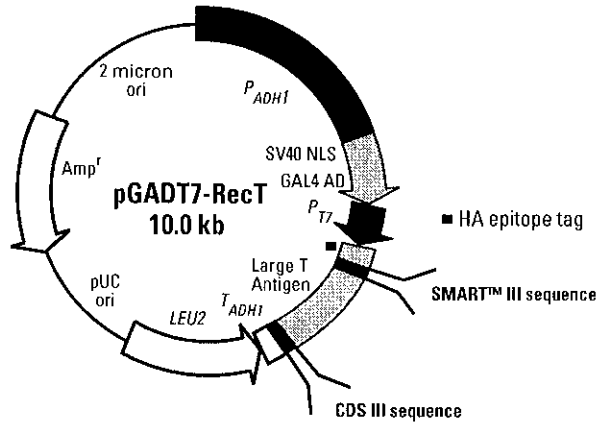


Figure 17. Map of pGADT7-RecT AD Control Vector. pGADT7-RecT is a product of recombination that encodes a fusion of the SV40 largeT antigen and the GAL4 AD. To generate this control, cotransform yeast with the SV40 LargeT PCR Fragment and pGADT7-Rec Cloning Vector (*Sma* I-linearized). Because the linearized vector shares sequence homology with the ends of the Large T PCR Fragment, these two components recombine via a double-crossover mechanism to produce the circular control plasmid pGADT7-RecT. The SV40 Large T DNA (GenBank Locus SV4CG) was derived from a plasmid referenced in Li & Fields (1993). PCR amplification was performed at Clontech.

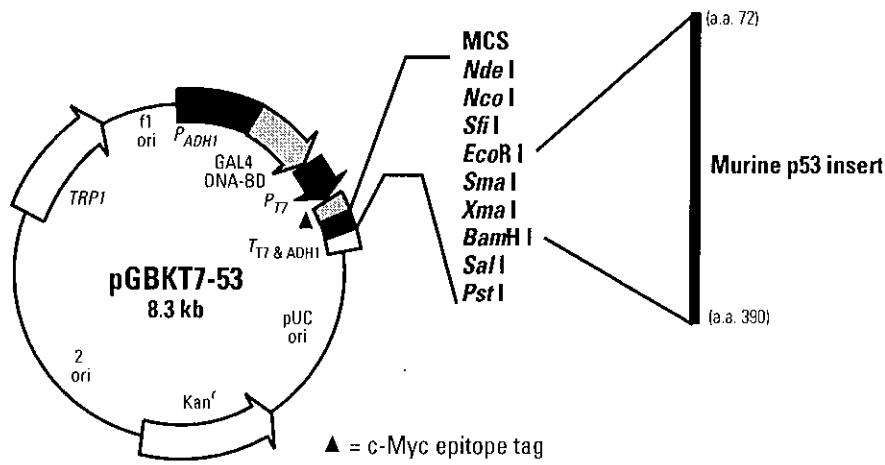
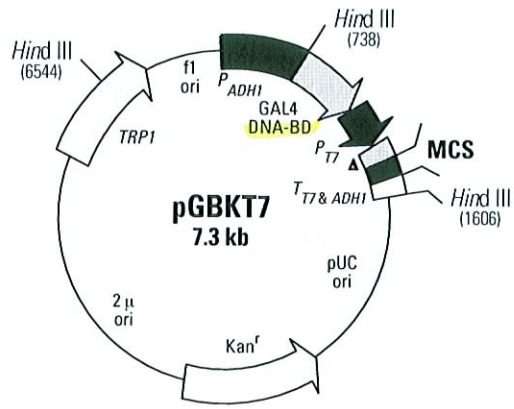
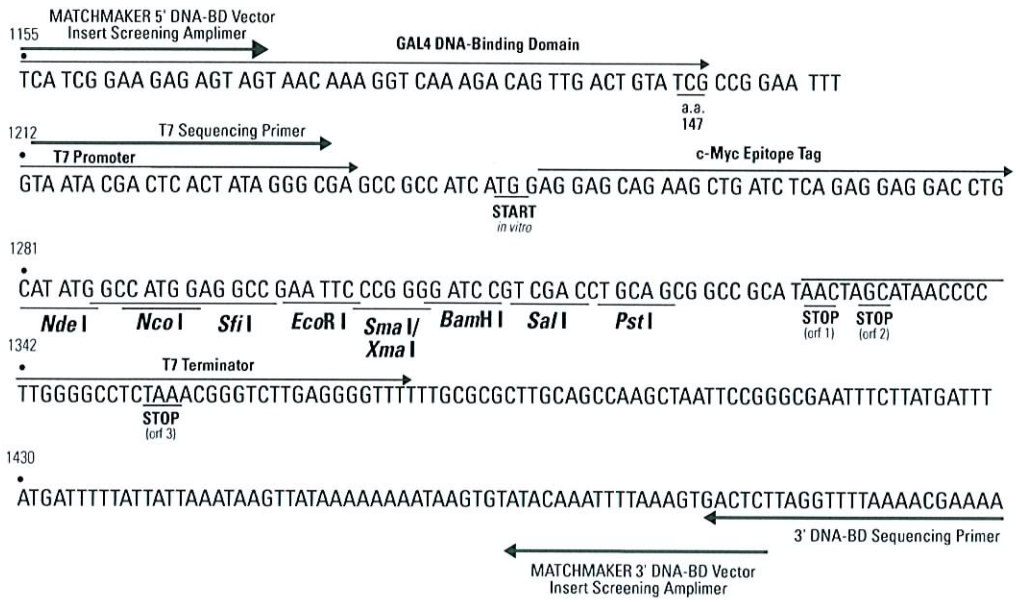


Figure 18. Map of pGBKT7-53 DNA-BD Control Vector. pGBKT7-53 is a positive control plasmid that encodes a fusion of the murine p53 protein (a.a. 72–390) and the GAL4 DNA-BD (a.a. 1–147). The murine p53 cDNA (GenBank Accession Cat. No. K01700) was cloned into pGBKT7 at the *Eco*R I and *Bam*H I sites. The p53 insert was derived from the plasmid described in Iwabuchi *et al.* (1993); plasmid modification was performed at Clontech. pGBKT7-53 has not been sequenced.



▲ c-Myc epitope tag



Restriction Map and Multiple Cloning Site (MCS) of pGBKT7. Unique restriction sites are in bold.

Description:

The pGBKT7 vector expresses proteins fused to amino acids 1–147 of the GAL4 DNA binding domain (DNA-BD). In yeast, fusion proteins are expressed at high levels from the constitutive *ADH1* promoter (P_{ADH1}); transcription is terminated by the T7 and *ADH1* transcription termination signals ($T_{T7\ \&\ ADH1}$). pGBKT7 also contains the T7 promoter, a c-Myc epitope tag, and a MCS. pGBKT7 replicates autonomously in both *E. coli* and *S. cerevisiae* from the pUC and 2 μ ori, respectively. The vector carries the Kan^r for selection in *E. coli* and the *TRP1* nutritional marker for selection in yeast. Yeast strains containing pGBKT7 exhibit a higher transformation efficiency than strains carrying other DNA-BD domain vectors (1).

(PR631431; published 30 March 2006)

Use:
pGBKT7 is the DNA-BD Vector included with MATCHMAKER Two-Hybrid System 3 (Cat. No. 630303). The MCS of pGBKT7 contains unique restriction sites in frame with the 3' end of the GAL4 DNA-BD for constructing fusion proteins with a bait protein. The bait protein is also expressed as a fusion to a c-Myc epitope tag. c-Myc tagged proteins can be identified with antibodies raised to this common epitope, eliminating the need to generate specific antibodies to new proteins. The T7 promoter is used for *in vitro* transcription and translation of the epitope tagged fusion protein. Note that the DNA-BD is not expressed during the *in vitro* transcription and translation reactions.

The MCS in pGBKT7 is compatible with those in pMyc-CMV and pHA-CMV, Clontech's epitope tagged mammalian expression vector set (Cat. No. 631604). As a result, the target gene can be shuttled into these vectors in order to confirm protein interactions *in vivo*.

Location of features:
- Truncated *S. cerevisiae ADH1* promoter (P_{ADH1}): 30–736
- GAL4 DNA binding domain (DNA-BD) polypeptide amino acids 1–147: 762–1202
- T7 RNA polymerase promoter: 1212–1235
- c-Myc epitope tag: 1248–1280
- Multiple Cloning Site: 1281–1334
- Transcription termination signals
  T7 terminator: 1335–1381
  ADH1 terminator: 1414–1610
- pUC plasmid replication origin: 1838–2636
- Kanamycin resistance gene: 4144–3222
- Yeast 2 μ replication origin: 4148–5493
- *TRP1* coding sequences
  promoter: 5559–6755
  gene: 6031–6705
- f1 bacteriophage origin of replication: 6756–29

Location of primers:
- T7 Sequencing Primer: 1213–1233
- 3' DNA-BD Sequencing Primer: 1510–1487

Propagation in *E. coli*:
- Suitable host strains: DH5α, DH10 & other general purpose strains
- Selectable marker: plasmid confers resistance to kanamycin (50 μg/ml) in *E. coli* hosts
- *E. coli* replication origin: pUC
- Copy number: ~500
- Plasmid incompatibility group: pMB1/Col E1

Propagation in *S. cerevisiae*:
- Suitable host strains: Y187(α), Y190(a), SFY526(a), CG1945(a), HF7c(a), or AH109(a)
- Selectable marker: *TRP1*
- *S. cerevisiae* origin: 2 μ

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 Clontech. This vector has not been completely sequenced.

Reference:
1. Louret, O. F., *et al.* (1997) *BioTechniques* **23:** 816–819.

Appendix D: Two-Hybrid Control Vector Information *continued*

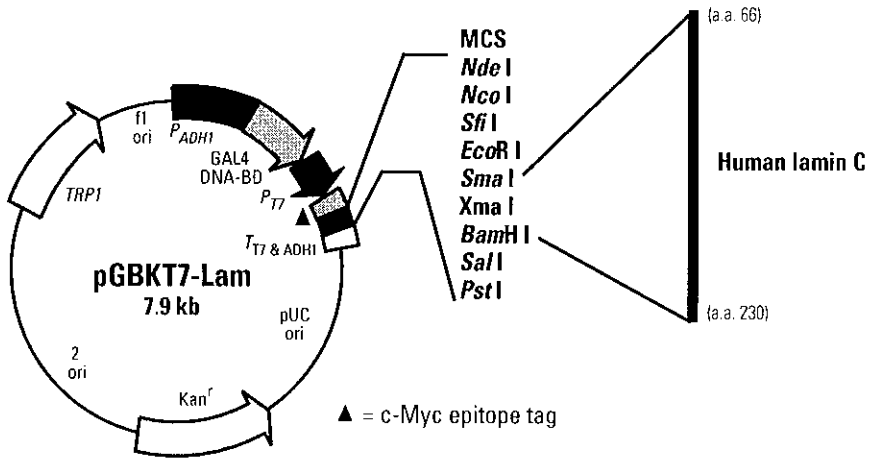


Figure 19. Map of pGBKT7-Lam DNA-BD Control Vector. pGBKT7-Lam is a negative control plasmid that encodes a fusion of the human lamin C protein (a.a. 66–230) and the GAL4 DNA-BD (a.a. 1–147). The lamin C cDNA insert (GenBank Accession Cat. No. M13451) was derived from the plasmid referenced in Bartel *et al.* (1993a). Plasmid modification was performed at Clontech. Yeast cotransformed with pGBKT7-Lam and pGADT7-RecT, provide a measure of the background that is due to false-positive two-hybrid interactions. pGBKT7-Lam has not been sequenced.

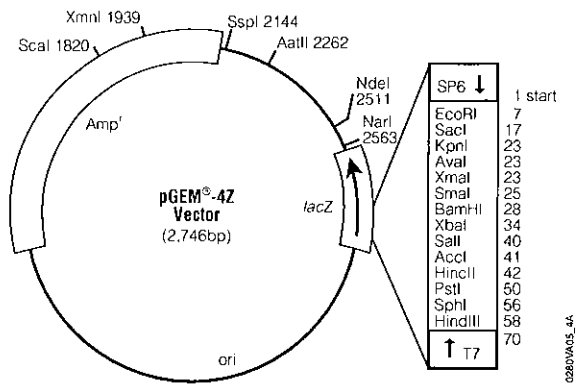


Figure 2. pGEM®-4Z Vector circle map and sequence reference points. The pGEM®-3Z and pGEM®-4Z Vectors are identical except for the orientation of the SP6 and T7 promoters.

pGEM®-4Z Vector sequence reference points:

SP6 RNA polymerase transcription initiation site	1
multiple cloning region	7–63
T7 RNA polymerase promoter (–17 to +3)	68–87
T7 RNA polymerase transcription initiation site	70
lac operon sequences	96–325; 2566–2726
binding site of pUC/M13 Reverse Sequencing Primer	106–122
lacZ start codon	110
lacZ operator	130–146
β-lactamase (Amp^r) coding region	1267–2127
binding site of pUC/M13 Forward Sequencing Primer	2686–2702
SP6 RNA polymerase promoter (–17 to +3)	2730–3

Specialized applications of the pGEM®-4Z Vector:

- Blue/white screening for recombinants.
- Transcription in vitro from dual-opposed promoters (For protocol information, please see the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016.)

Note: All Promega technical literature is available at: **www.promega.com/tbs**

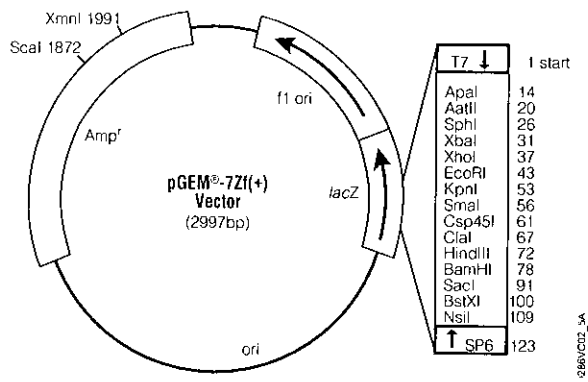


Figure 2. pGEM®-7Zf(+) Vector circle map and sequence reference points. The pGEM®-7Zf(+) and pGEM®-7Zf(–) Vectors are identical except for the orientation of the f1 origin. Use the T7 Promoter Primer or pUC/M13 Forward Primer to sequence ssDNA produced by the pGEM®-7Zf(+) Vector.

pGEM®-7Zf(+) Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	123
T7 RNA polymerase promoter (-17 to +3)	2981–3
SP6 RNA polymerase promoter (-17 to +3)	121–140
multiple cloning region	10–110
binding site of pUC/M13 Reverse Sequencing Primer	158–174
*lac*Z start codon	162
lac operon sequences	2818–2978; 148–377
lac operator	182–198
β-lactamase (Amp^r) coding region	1319–2179
phage f1 region	2362–2817
binding site of pUC/M13 Forward Sequencing Primer	2938–2954

Specialized applications of the pGEM®-7Zf(+) Vector:

- Used with the Erase-a-Base® System
- ssDNA production
- Blue/white screening for recombinants
- Transcription in vitro from dual-opposed promoters (For protocol information, please request the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016.)
- Translation in vitro (For protocol information, please request the *TNT® Quick Coupled Transcription/Translation System Technical Manual*, #TM045.)

Note: All Promega technical literature is available on the Internet at: **www.promega.com**

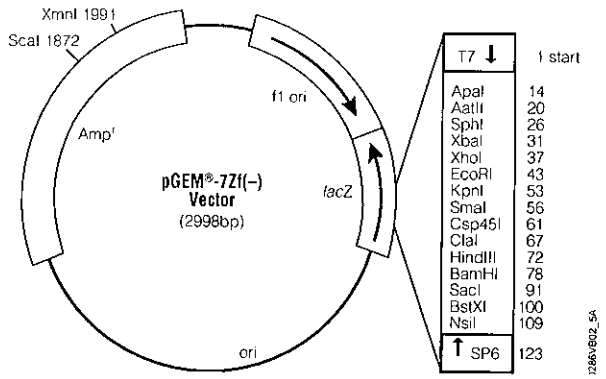


Figure 2. pGEM®-7Zf(-) Vector circle map and sequence reference points. The pGEM®-7Zf(+) and pGEM®-7Zf(-) Vectors are identical except for the orientation of the f1 origin. Use the SP6 Promoter Primer (Cat.# Q5011) or pUC/M13 Reverse Primer (Cat.# Q5401) to sequence ssDNA produced by the pGEM®-7Zf(-) Vector.

pGEM®-7Zf(-) Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
SP6 RNA polymerase transcription initiation site	123
T7 RNA polymerase promoter (-17 to +3)	2982-3
SP6 RNA polymerase promoter (-17 to +3)	121-140
multiple cloning region	10-110
binding site of pUC/M13 Reverse Sequencing Primer	158-174
lacZ start codon	162
lac operon sequences	2819-2979; 148-377
lac operator	182-198
β-lactamase (Amp^r) coding region	1319-2179
phage f1 region	2363-2818
binding site of pUC/M13 Forward Sequencing Primer	2939-2955

Specialized applications of the pGEM®-7Zf(-) Vector:

- ssDNA production
- Blue/white screening for recombinants
- Transcription in vitro from dual-opposed promoters (For protocol information, please request the *Riboprobe® in vitro Transcription Systems Technical Manual*, #TM016.)

Note: All Promega technical literature is available on the Internet at: **www.promega.com**

5.C. Sequence and Multi-Cloning Site of the pGEM®-T Easy Vector

The sequence of the pGEM®-T Easy Vector is available at:
www.promega.com/vectors/
The pGEM®-T Easy Vector has been linearized at base 60 with EcoRV and a T added to both 3´-ends. The EcoRV site will not be recovered upon ligation of the vector and insert.

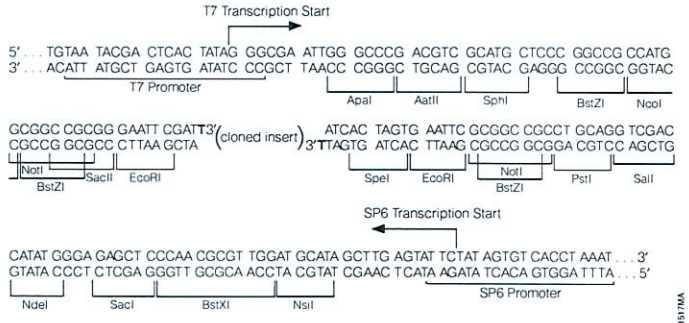
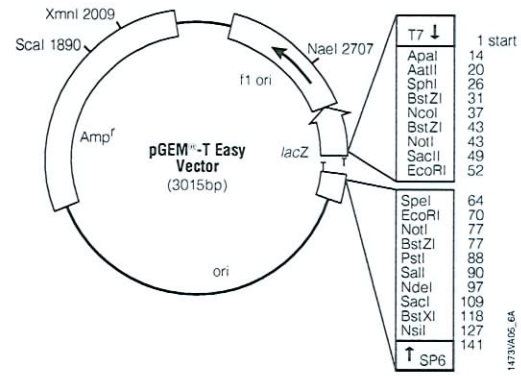


Figure 2. The promoter and multiple cloning sequence of the pGEM®-T Easy Vector. The top strand shown corresponds to the RNA synthesized by T7 RNA polymerase. The bottom strand corresponds to the RNA synthesized by SP6 RNA polymerase.

More PCR Cloning Resources are available in the Cloning Chapter of the Protocols and Applications Guide at:
www.promega.com/paguide

5.D. pGEM®-T Easy Vector Map and Sequence Reference Points



pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
lacZ start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

Note: Inserts can be sequenced using the SP6 Promoter Primer (Cat.# Q5011), T7 Promoter Primer (Cat.# Q5021), pUC/M13 Forward Primer (Cat.# Q5601), or pUC/M13 Reverse Primer (Cat.# Q5421).

Note: A single digest with BstZI (Cat.# R6881), EcoRI (Cat.# R6011) or NotI (Cat.# R6431) will release inserts cloned into the pGEM®-T Easy Vector. Double digests can also be used to release inserts.

GE Healthcare

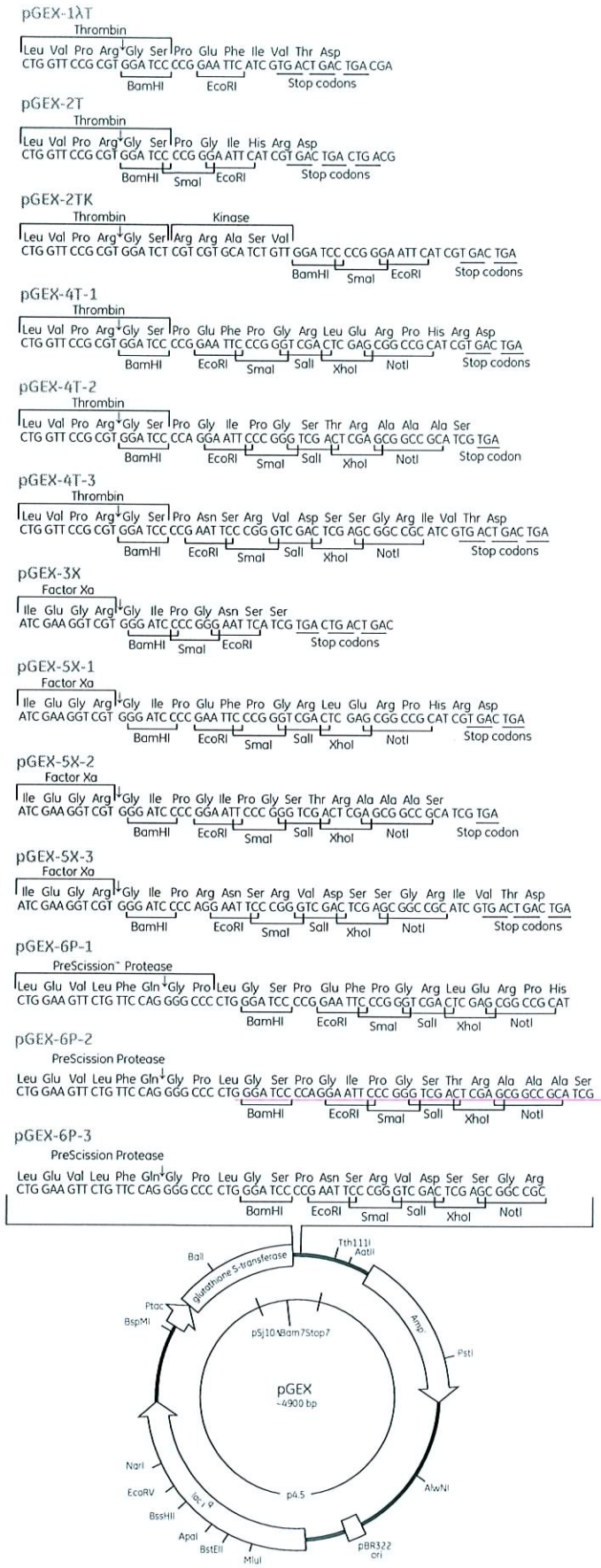
pGEX vectors, GST Gene Fusion System

Map of the glutathione S-transferase fusion vectors showing reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

Ordering information

Product	Quantity	Code no.
pGEX-1λT EcoRI/BAP	5 µg	28-9546-56
pGEX-2T	25 µg	28-9546-53
pGEX-2TK	25 µg	28-9546-46
pGEX-3X	25 µg	28-9546-54
pGEX-4T-1	25 µg	28-9545-49
pGEX-4T-2	25 µg	28-9545-50
pGEX-4T-3	25 µg	28-9545-52
pGEX-5X-1	25 µg	28-9545-53
pGEX-5X-2	25 µg	28-9545-54
pGEX-5X-3	25 µg	28-9545-55
pGEX-6P-1	25 µg	28-9546-48
pGEX-6P-2	25 µg	28-9546-50
pGEX-6P-3	25 µg	28-9546-51

Do you want to learn more? Read the GST Gene Fusion System Handbook (18-1142-75). Please contact your local GE Healthcare representative for a printed copy.



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For local office contact information, visit
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GE Healthcare Bio-Sciences KK
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Japan

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28-9191-62 AC 07/2009

III.D. pGL3-Control Vector

The pGL3-Control Vector contains SV40 promoter and enhancer sequences, resulting in strong expression of *luc+* in many types of mammalian cells. This plasmid is useful in monitoring transfection efficiency, in general, and is a convenient internal standard for promoter and enhancer activities expressed by pGL3 recombinants.

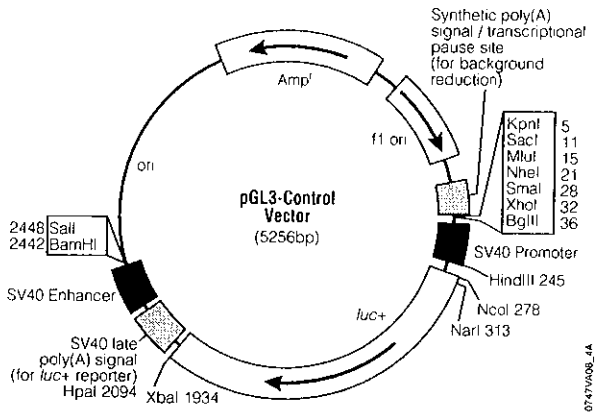


Figure 4. pGL3-Control Vector circle map. Additional description: *luc+*, cDNA encoding the modified firefly luciferase; Amp^r, gene conferring ampicillin resistance in *E. coli*; f1 ori, origin of replication derived from filamentous phage; ori, origin of plasmid replication in *E. coli*. Arrows within *luc+* and the Amp^r gene indicate the direction of transcription; the arrow in f1 ori indicates the direction of ssDNA strand synthesis.

pGL3-Control Vector Sequence Reference Points:

Multiple cloning region	1–41
Promoter	48–250
Luciferase gene (*luc+*)	280–1932
GLprimer2 binding site	281–303
SV40 late poly(A) signal	1964–2185
Enhancer	2205–2441
RVprimer4 binding site	2499–2518
ColE1-derived plasmid replication origin	2756
β-lactamase gene (Amp^r)	3518–4378
f1 origin	4510–4965
upstream poly(A) signal	5096–5249
RVprimer3 binding site	5198–5217

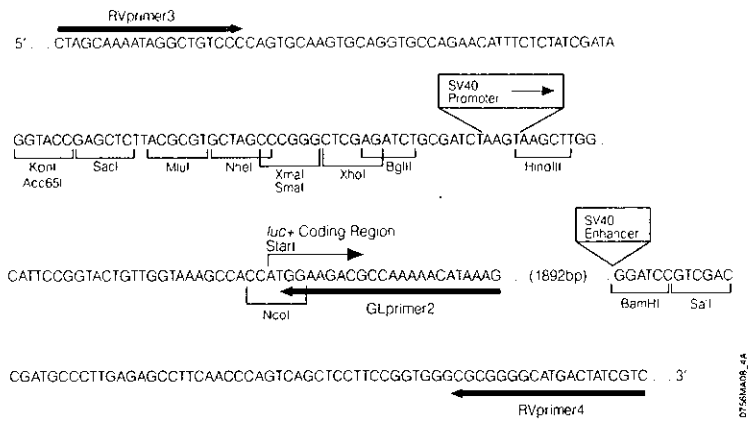
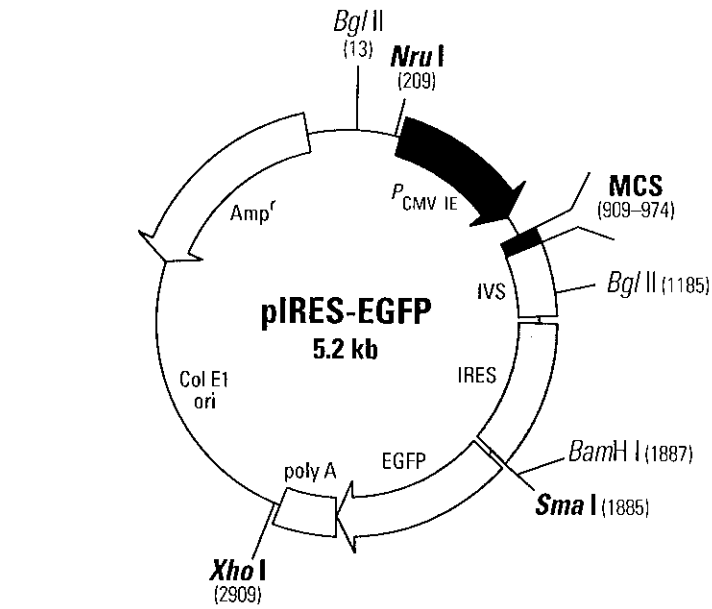


Figure 5. pGL3 Vector multiple cloning regions. Shown are the upstream and downstream cloning sites and the locations of the sequencing primers (GLprimer2, RVprimer3 and RVprimer4). The large primer arrows indicate the direction of sequencing. The positions of the promoter (in the pGL3-Promoter and pGL3-Control Vectors) and the enhancer (in the pGL3-Enhancer and pGL3-Control Vectors) are shown as insertions into the sequence of the pGL3-Basic Vector. (Note that the promoter replaces four bases [AAGT] of the pGL3-Basic Vector.) The sequence

pIRES-EGFP Vector Information PT3157-5

GenBank Accession #: Submission in progress. Catalog #6064-1



Restriction Map and Multiple Cloning Site (MCS) of pIRES-EGFP Vector. Unique restriction sites are in bold. The *Cla* I site (*) in the MCS is methylated in the DNA provided by CLONTECH. If you wish to digest the vector with this enzyme, you will need to transform the vector into a *dam*⁻ host and make fresh DNA. The *Xho* I site can be used to linearize the vector before transfection when generating stable cell lines.

Description:

pIRES-EGFP contains the internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV) between the MCS and the EGFP (enhanced green fluorescent protein) coding region. This permits both the gene of interest (cloned into the MCS) and the EGFP gene to be translated from a single bicistronic mRNA. pIRES-EGFP is designed for the efficient selection (by flow cytometry or other methods) of transiently transfected mammalian cells expressing EGFP and another protein of interest. To optimize the selection of cells expressing high levels of the protein of interest, pIRES-EGFP utilizes a partially disabled IRES sequence (1). This attenuated IRES leads to a reduced rate of translation initiation at the EGFP start codon relative to that of the cloned gene. This enables you to select those cells in which the mRNA, and hence the target protein, is produced at high levels to compensate for a suboptimal rate of translation of EGFP. This vector can also be used to express EGFP alone or to obtain stably transfected cell lines without time-consuming drug and clonal selection.

EGFP is a red-shifted variant of wild-type GFP that has been optimized for brighter fluorescence and higher expression in mammalian cells. (Excitation maximum = 488 nm; emission maximum = 509 nm.) EGFP encodes the GFPmut1 variant (2), which contains the amino acid substitutions Phe-64 to Leu and Ser-65 to Thr. These mutations increase the brightness and solubility of GFP, primarily due toimproved protein folding properties and efficiency of chromophore formation (2, 3). EGFP also contains an open reading frame comprised almost entirely of preferred human codons (4). This leads to more efficient translation and, hence, higher expression levels in eukaryotic cells, relative to wt GFP.

(PR7Y616)

pIRES-EGFP was derived from pIRES1*neo* (#6060-1; originally described as pCIN4 by Rees *et al.*; 1) by replacing the *neo* gene downstream of the IRES sequence with the EGFP coding region. The IRES sequence permits the translation of two open reading frames from one messenger RNA (5, 6). The expression cassette of pIRES-EGFP contains the human cytomegalovirus (CMV) major immediate early promoter/enhancer followed by a multiple cloning site (MCS), a synthetic intron (IVS) known to enhance the stability of the mRNA (7), the EMCV IRES followed by the EGFP coding region, and the polyadenylation signal of the bovine growth hormone. Ribosomes can enter the bicistronic mRNA at the 5' end to translate the gene of interest and at the ECMV IRES to translate the EGFP gene.

Location of features:

- $P_{CMV\ IE}$ promoter: 232–820
- Multiple cloning site (MCS): 909–974
- Synthetic intron (IVS): 974–1269
- Internal ribosome entry site (IRES) of the encephalomyocarditis virus (ECMV): 1299–1884
- Enhanced green fluorescent protein (EGFP) gene: 1905–2621
- Fragment containing the bovine growth hormone poly-A signal: 2636–2913
- Col E1 origin of replication: 3343–4016
- Ampicillin resistance gene: 5026–4168

Propagation in *E. coli*

- Suitable host strains: DH5α and other general purpose strains.
  Note: If you wish to digest the vector with *Cla* I, you will need to transform a *dam*⁻ *E. coli* host and obtain a fresh plasmid preparation.
- Selectable marker: plasmid confers resistance to ampicillin (50 μg/ml) on *E. coli* hosts.

Transfection of mammalian cells

- To promote unrearranged integration in the host genome, we recommend linearizing the vector before transfection. However, prelinearization of the vector is not required to generate stable cell lines.
- Note that prolonged exposure to high levels of EGFP may be slightly toxic to some types of mammalian cells.

References:

1. Rees, S., *et al.* (1996) *Biotechniques* **20**:102–110.
2. Cormack, B., *et al.* (1996) *Gene* **173**:33–38.
3. Yang, T. T., *et al.* (1996) *Nucleic Acids Res.* **24**:4592–4593.
4. Haas, J., *et al.* (1996) *Curr. Biol.* **6**:315–324.
5. Jackson, R. J., *et al.* (1990) *Trends Biochem.* **15**:477–483.
6. Jang, S. K., *et al.* (1988) *J. Virol.* **62**:2636–2643.
7. Huang, M. T. F. & Gorman, C. M. (1990) *Nucleic Acids Res.* **18**(4):937–947.

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 CLONTECH. This vector has not been completely sequenced.

Certificate of Analysis

pmirGLO Dual-Luciferase miRNA Target Expression Vector:

Cat.#	Size
E1330	20µg

Cat.# E1330 contains:

Part No.	Name	
E133A	pmirGLO Vector	20µg
C838A	Oligo Annealing Buffer	1ml

Part# 9PIE133
Revised 10/09

Description: The pmirGLO Dual-Luciferase miRNA Target Expression Vector[a-c] is designed to quantitatively evaluate microRNA (miRNA) activity by the insertion of miRNA target sites 3′ of the firefly luciferase gene (*luc2*). These target sites can be introduced by cloning putative miRNA binding sites alone, or the 3′ untranslated region (UTR) of a gene of interest, to study the influence of these sites on transcript stability and activity. Firefly luciferase is the primary reporter gene; reduced firefly luciferase expression indicates the binding of endogenous or introduced miRNAs to the cloned miRNA target sequence. This vector is based on Promega dual-luciferase technology, with firefly luciferase (*luc2*) used as the primary reporter to monitor mRNA regulation and *Renilla* luciferase (*hRluc-neo*) acting as a control reporter for normalization and selection. This vector contains the following features:

- Human phosphoglycerate kinase (PGK) promoter provides low translational expression, which is advantageous when reduction of signal is the desired response. The PGK promoter is a nonviral universal promoter, which functions across cell lines (yeast, rat, mouse and human).
- Firefly luciferase reporter gene (*luc2*) inversely reports miRNA activity in mammalian cells.
- Multiple cloning site (MCS) is located 3′ of the firefly luciferase reporter gene (*luc2*).
- Humanized *Renilla* luciferase-neomycin resistance cassette (*hRluc*-neo) is used as a control reporter for normalization of gene expression and stable cell line selection.
- Amp^r gene allows bacterial selection for vector amplification.
- SV40 late poly(A) signal sequence is positioned downstream of *luc2* to provide efficient transcription termination and mRNA polyadenylation.
- Synthetic poly(A) signal/transcription stop site.

Concentration: 1µg/µl in 10mM Tris-HCl, 1mM EDTA; final pH 7.4.

GenBank® Accession Number: FJ376737.

Storage Conditions: See the storage temperature and expiration date on the Product Information Label.

Quality Control Assays

Functional Assays

Identity Assay: The vector has been sequenced completely and has 100% identity with the published sequence available at: **www.promega.com/vectors/**

Restriction Digestion: The functional purity of this vector DNA is verified by complete digestion with restriction enzymes at the optimal temperature for 1 hour. Samples are examined by agarose gel electrophoresis, comparing cut and uncut vector DNA with marker DNA.

Contaminant Assays

Contaminating Nucleic Acids: RNA, single-stranded DNA and chromosomal DNA are not evident in specified quantities of this vector as determined by agarose gel electrophoresis.

Nuclease Assay: Following incubation of 1µg of this vector in Restriction Enzyme Buffer at 37°C for 16–24 hours, no evidence of nuclease activity is detected by agarose gel electrophoresis.

Physical Purity: $A_{260}/A_{280} \geq 1.80$, $A_{260}/A_{250} \geq 1.05$.

Signed by: J. Stevens

J. Stevens, Quality Assurance

Promega

Promega Corporation

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[b]U.S. Pat. No. 5,670,356.
[c]Australian Pat. No. 2001 285278 and other patents pending.
[d]The method of recombinant expression of *Coleoptera* luciferase is covered by U.S. Pat. Nos. 5,583,024, 5,674,713 and 5,700,673. A license (from Promega for research reagent products and from The Regents of the University of California for all other fields) is needed for any commercial sale of nucleic acid contained within or derived from this product.
[e]Licensed from University of Georgia Research Foundation, Inc., under U.S. Pat. Nos. 5,292,658, 5,418,155, Canadian Pat. No. 2,105,984 and related patents.

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Promega

Features List and Map for the pmirGLO Vector

SV40 late poly(A) signal	106–327
SV40 early enhancer/promotor	426–844
hRluc-neo fusion protein coding region	889–2664
Synthetic polyadenylation signal	2728–2776
β-lactamase (Amp^r) coding region	3037–3897
*Col*E1-derived plasmid origin of replication	4052–4088
Human phosphoglycerate kinase promoter	5094–5609
luc2 reporter gene	5645–7297
Multiple cloning site (MCS, Figure 1)	7306–7350

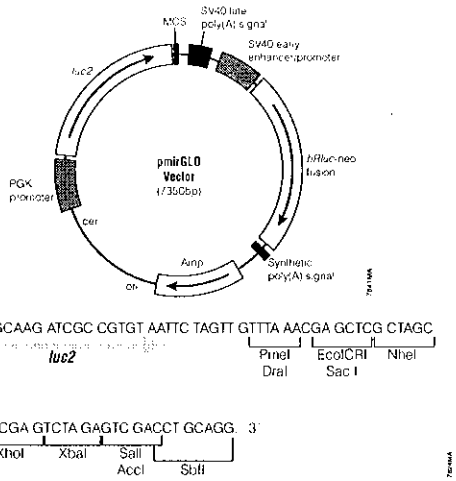

Figure 1. pmirGLO Vector multiple cloning site.

I. Sample Protocol

A. Vector Cloning

1. Design oligonucleotides: Order oligonucleotide pairs that contain the desired miRNA target region and, when annealed and ligated into the pmirGLO Vector, result in the miRNA target region in the correct 5′ to 3′ orientation. Insure that the overhangs created by oligonucleotide annealing are complementary to those generated by restriction enzyme digestion of the pmirGLO Vector in Step 2. Add an internal restriction site to your oligonucleotides for clone confirmation (e.g., NotI in Figure 3 creates a ~125bp insert when digested with NotI because of a NotI site at position 93 in the vector).
2. Digest vector: Linearize the pmirGLO Vector with the appropriate restriction enzymes to generate overhangs that are complementary to the annealed oligonucleotide overhangs.
3. Anneal oligonucleotides: Dilute both oligonucleotides (supplied by user) to 1µg/µl. Combine 2µl of each oligonucleotide with 46µl of Oligo Annealing Buffer. Heat at 90°C for 3 minutes, then transfer to a 37°C water bath for 15 minutes. Use the annealed oligonucleotides immediately, or store at –20°C.

B. Ligation and Transformation

1. Dilute annealed oligonucleotides 1:10 in nuclease-free water to a final concentration of 4ng/µl per oligonucleotide. Ligate 4ng of annealed oligonucleotides and 50ng of linearized vector using a standard ligation protocol. Transform ligated pmirGLO Vector using high-efficiency JM109 competent cells (e.g., Cat.# L2001).
2. Select clones on ampicillin-containing plates, then select clones containing the oligonucleotides by digesting miniprep-purified DNA (e.g., purified using the PureYield™ Plasmid Miniprep System, Cat.# A1221) using the unique restriction site in the oligonucleotide pair. The purified plasmid DNA can be transfected directly or expanded to generate more DNA.

Additional information about annealing, ligation, transformation and oligonucleotide design can be found in the *GeneClip™ U1 Hairpin Cloning Systems Technical Manual*, #TM256, which is available at: **www.promega.com/tbs/**

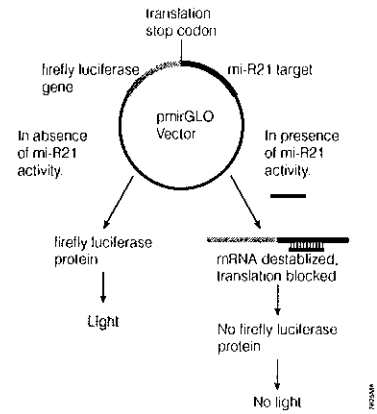

Figure 2. Mechanism of action of the pmirGLO Vector.

	PmeI	NotI Internal site	miR-21 target sequence	XbaI
miR21 sense, PmeI and XbaI	5′ **AAAC** TA	GCGGCCGC	TAGT TCAACATCAG*TC*TGATAAGCTA	**T** 3′
miR21 mismatch sense, PmeI and XbaI	5′ **AAAC** TA	GCGGCCGC	TAGT TCAACATCAG*AA*GATAAGCTA	**T** 3′

	XbaI	miR-21 target sequence	NotI internal site	PmeI
miR21 antisense, PmeI and XbaI	5′ **CTAGA**	TAGCTTATC*AGA*CTGATGTTGA ACTA	GCGGCCGC	TA **GTTT** 3′
miR21 mismatch antisense, PmeI and XbaI	5′ **CTAGA**	TAGCTTATC*TT*CTGATGTTGA ACTA	GCGGCCGC	TA **GTTT** 3′

Figure 3. Sample oligonucleotides for miR21.

C. An Example of Detecting miR21 Activity Using the pmirGLO Vector:miR-21 Construct

An overview describing the use of the pmirGLO Vector to interrogate endogenous miR21 microRNA is shown in Figure 2.

The presence of broadly endogenous microRNA miR21 was monitored in HeLa cells. Constructs contained either an exact match to the 21bp miR21 target sequence or a mismatched version of that target site (1) as well as PmeI, XbaI and NotI restriction sites (Figure 3; mismatched sequence is in italics). Twenty-four hours after transfection with the miR21 pmirGLO Vector constructs, cells were analyzed for luciferase activity using the Dual-Glo® Luciferase Assay System (Cat.# E2920) and a MicroLumatPlus LB96V luminometer (Berthold). Normalized firefly luciferase activity (firefly luciferase activity/*Renilla* luciferase activity) for each construct was compared to that of the pmirGLO Vector no-insert control. For each transfection, luciferase activity was averaged from six replicates.

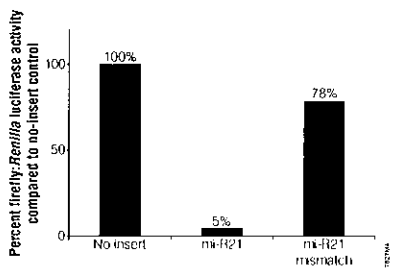

Figure 4. Normalized luciferase activity using the pmirGLO Vector with an miR21 target sequence.

II. Reference

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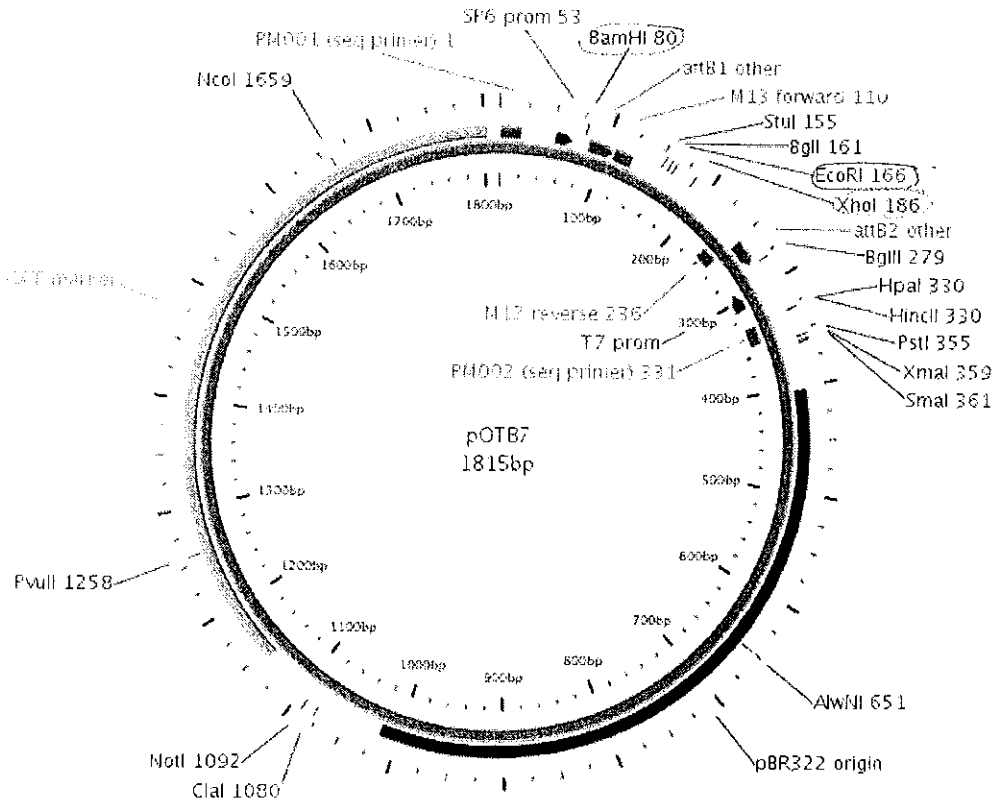
Part# 9PIE133
Printed in USA Revised 10/09

Berkeley Drosophila Genome Project

BDGP Resources

pOTB7 Vector




```
BamHI                                                    M13 for
ACTCGAAGGATCCACAAGTTTGTACAAAAAAGCAGGCTTGTAAAACGACG
AGTGCTTCCTAGGTGTTCAAACATGTTTTTTCGTCCGAACATTTTGCTGC
     I-CeuI                                  BglI      EcoRI
GCCAGTAACTATAACGGTCCTAAGGTAGCGAGGCCTGGGTGGCGAATTCC
CGGTCATTGATATTGCCAGGATTCCATCGCTCCGGACCCACCGCTTAAGG
   SpeI          XhoI                  I-SceI
CTTACTAGTTTCCCTCGAGGCATCTATGTCGGGTGCGGAGAAAGAGGTAA
GAATGATCAAAGGGAGCTCCGTAGATACAGCCCACGCCTCTTTCTCCATT

TGAAATGGCACATGGTCATAGCTGTTTCCTGACCCAGCTTTCTTGTACAA
ACTTTACCGTGTACCAGTATCGACAAAGGACTGGGTCGAAAGAACATGTT
  BglI                                  M13
                                        Reverse
AGTGGTAGATCTGC
TCACCATCTAGACG
```

The pOTB7 plasmid began with the pOT2A backbone. A 204bp fragment was designed to facilitate the use of the **Gateway** expression system from Life Technologies. Specifically:
An attB1 site is between the BamHI and M13 forward sites. An attB2 site is between BglII and M13 reverse sites.
The basic layout of the insert is shown above. The 204bp insert was ligated into the existing XhoI and EcoRI sites within the pOT2A vector. The XhoI site was destroyed and the EcoRI has been changed to a BglII site. The HindIII site in pOT2A was replaced by a NotI site. It is important to note that the EcoRI and XhoI sites are reversed with regard to the Sp6 and T7 sequencing primers compared to the pOT2A vector.

We sequence the 5' ESTs using the M13 Forward primer and the 3' ESTs using the M13 Reverse primer.

M13 forward (-21) 18-mer

TGTAA AACGA CGGCC AGT

M13 reverse 17-mer
CAGGA AACAG CTATG AC

>pOTB7, 1815 bases, 2153 checksum.
CGTTAGAACGCGGCTACAATTAATACATAACCTTATGTATCATACACATA
CGATTTAGGTGACACTATAGAACTCGAAGGATCCACAAGTTTGTACAAAA
AAGCAGGCTTGTAAAACGACGGCCAGTAACTATAACGGTCCTAAGGTAGC
GAGGCCTGGGTGGCGAATTCCCTTACTAGTTTCCCTCGAGGCATCTATGT
CGGGTGCGGAGAAAGAGGTAATGAAATGGCACATGGTCATAGCTGTTTCC
TGACCCAGCTTTCTTGTACAAAGTGGTAGATCTGCCGGTCTCCCTATAGT
GAGTCGTATTAATTTCGATAAGCCAGGTTAACCTGCATTAATGAATCGGC
TGCAGTACCCGGGAATTTAACCCGCCTAATGAGCGGGCTTTTTTTTGTGA
TCCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTT
GCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAG
AGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATA
CCAAATACTGTTCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAA
CTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGG
CTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGA
TAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCAC
ACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGC
GTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGG
TATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCC
AGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCT
GACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGG
AAAAACGCCAGCAACGCGGATCACAACAAAAAGCCCGCTCATTAGGCGGG
CTAAATTCTCATGTTTGACAGCTTATCATCGATAAGCTAGCGGCCGCTAG
CTTTAATGAGTTATCGAGATTTTCAGGAGCTAAGGAAGCTAAAATGGAGA
AAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAA
GAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATAACCAGAC
CGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGC
ACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGATGAATGCT
CATCCGGAGTTCCGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGA
TAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAAACGTTTT
CATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATA
TATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATTTCCCTAA
AGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTT
TCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTTCGCCCCC
GTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCC
GCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCATGTCGGCA
GAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCG
TAATTGGTACGTCGA

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Last updated: 16 January 2009, Size: 3.5K

Twenty μg total cell RNA was hybridized with [^{32}P]DNA in 0.25 M NaCl, 0.32 M Hepes pH 7.6, 3 mM EDTA. Reactions were incubated for 16 h at 65°C. The hybrids were digested with 100 U S_1 (BRL) in 4 mM $ZnSO_4$, 30 mM NaOAc pH 4.6, 0.25 M NaCl and the protected fragments were resolved on 6% acrylamide/urea gels.

Northern blots were performed as described in Maniatis *et al.* (1982).

Photomicroscopy

For visualization of cell nuclei and spores cells were fixed in 70% ethanol for 18 h, rinsed in 50 mM sodium citrate pH 7.0, and incubated in 50 μg/ml RNase at 37°C for 1 h. Propidium iodide (Sigma) was added to 2μg/ml and the cells were visualized under combined phase and fluorescence microscopy. This procedure highlights cell nuclei. Fully mature spores fluoresce with exaggerated intensity because RNase fails to penetrate and degrade the RNA within these structures.

Expression of mei3$^+$ in E. coli

The plasmid *pAR3038* is a derivative *pBR322* carrying 23 bp of the promoter of gene 10 of the bacteriophage *T7*. The *T7* promoter is flanked by *Bgl*II and *Eco*RV restriction sites and is adjacent to a unique *Nde*I site that is suitable for the insertion of target genes (F.W.Studier, personal communication). The plasmid *pRK172* was derived from *pAR3038* by digestion with *Eco*RI (position 4361 in *pBR322*) and *Eco*RV followed by flush ending with the Klenow fragment of DNA polymerase and blunt-end ligation. The resulting plasmid, which has a deletion in the promoter region of the tetracycline resistance gene was further digested with *Pvu*II (position 2066 in *pBR322*) and *Bgl*II and blunt-end ligated in order to remove the copy number control region of *pBR322*. The unique *Bam*HI site located downstream of the *T7* promoter region was filled in with Klenow polymerase and an *Eco*RI linker was added at this site to give *pRK172* (R.Kostriken, personal communication). *pRK172* therefore contains the *T7* gene 10 promoter but is propagated at very high copy number in *E. coli* due to removal of the pBR322 copy number control region.

An *Nde*I site was created at the initiating methionine of the *mei3*$^+$ gene by oligonucleotide mutagenesis of the plasmid *pmei3.14'*. This consists of pUC118 carrying the *mei3*$^+$ gene as an *Eco*RV restriction fragment (Figure 1). Single-stranded DNA was obtained from this plasmid and annealed with the 23 mer 5'TTTCTACTTCTACATATGAGCTC3'. Mutagenesis was as described (Zoller and Smith, 1984) and resulted in *pmei3.14'(Nde)* which is identical to *pmei3.14'* except that it contains an *Nde*I site (5'CATATG3') at the initiating methionine of the gene. *mei3*$^+$ was cloned as a *NdeI/Eco*RI fragment into pRK172 to give *pME13.18*.

The *E. coli* strain BL21(DE3) was used for expression of *mei3*$^+$ protein. The strain carries a chromosomal copy of the bacteriophage T7 RNA polymerase gene under the control of the *lac* UV5 promoter (Studier and Moffatt, 1986). The plasmid *pME13.18* was introduced into this strain and ampicillin-resistant colonies were obtained. Individual transformants were grown at 37°C in 10 ml L Broth containing 50 μg/ml ampicillin. When the culture reached an OD_{660} of 0.2, glycerol was added to a final concentration of 15% and the cells were frozen at −70°C. For production of the *mei3*$^+$ protein, frozen cells were thawed and 10 λ innoculated into 10 ml M9 media (Miller, 1972) containing ampicillin. The culture was induced with 0.4 mM IPTG (Sigma) when attaining an OD_{660} of 0.4 and cells were harvested by centrifugation 3 h after induction. Cell pellets were stored frozen at −70°C.

Purification of mei3$^+$ protein

Frozen pellets (10-ml cells) were thawed on ice and resuspended in 1.0 ml 50 mM Tris−HCl pH 7.8, 2 mM DTT, 5 mM EDTA, 2 mM benzamidine HCl, 2 mM PMSF 10% glycerol, 0.3% Triton X100 and 0.6 mg/ml lysozyme (Sigma). After incubation on ice for 30 min, cells were sonicated for four 3-s intervals. The extract was clarified by centrifugation at 10 000 *g* for 20 min. Approximately 60% of the *mei3*$^+$ protein was found in the insoluble pellet and the material was solubilized in 1% sodium deoxycholate. The solubilized material consisted primarily of *mei3* protein and one other major contaminant. The contaminant was removed by DE52 cellulose (Whatman) chromatography.

Preparation of monoclonal antibodies

BalbC female mice (National Cancer Institute) were immunized with *mei3*$^+$ protein according to the following schedule. The first i.p. injection contained 50 μg *mei3*$^+$ protein in complete Freund's adjuvant. This was followed at 2-week intervals by further i.p. injections. Three weeks following the last injection, mice were immunized with 50 μg *mei3*$^+$ protein i.v. Mice were sacrificed 3 days after the final injection and spleen cells were fused in the presence of polyethylene glycol to NS-1 myeloma cells by standard techniques (Galfre *et al.*, 1977).

by vortexing for 3 min. After 30 min incut
were collected by centrifugation. The pel
saturated ether and resolubilized in 5% :
diluted 1:1 in 2 × sample buffer (Laemmli,
were electrophoresed on a 7.5−15.0% g
1970). Proteins were transferred to nitroce
as described in Burnette (1981). Following
blocked in 3% ovalbumin (Sigma) in phc
for 2 h. Papers were washed in 150 mM
0.05 NP-40. 50 mM Tris 7.5 (NET) for
incubated with tissue culture supernatan
(N−8) for 1 h at room temperature. After
was incubated with 20 μCi ^{125}I rabbit ant
in PBS, 1% BSA for 30 min at room ten
NET, filters were dried and exposed to

Acknowledgements

We thank Jeff Vieira for providing the pla
Studier for providing the BL21(DE3) stra
the *adh* promoter, and Mark Zoller for synt
of Carmelita Bautista with the construct
gratefully acknowledged. Jean Roberts, L
thanked for their assistance with preparati
work was supported by NIH grant GM3
postdoctoral fellowship to Maureeen Mc

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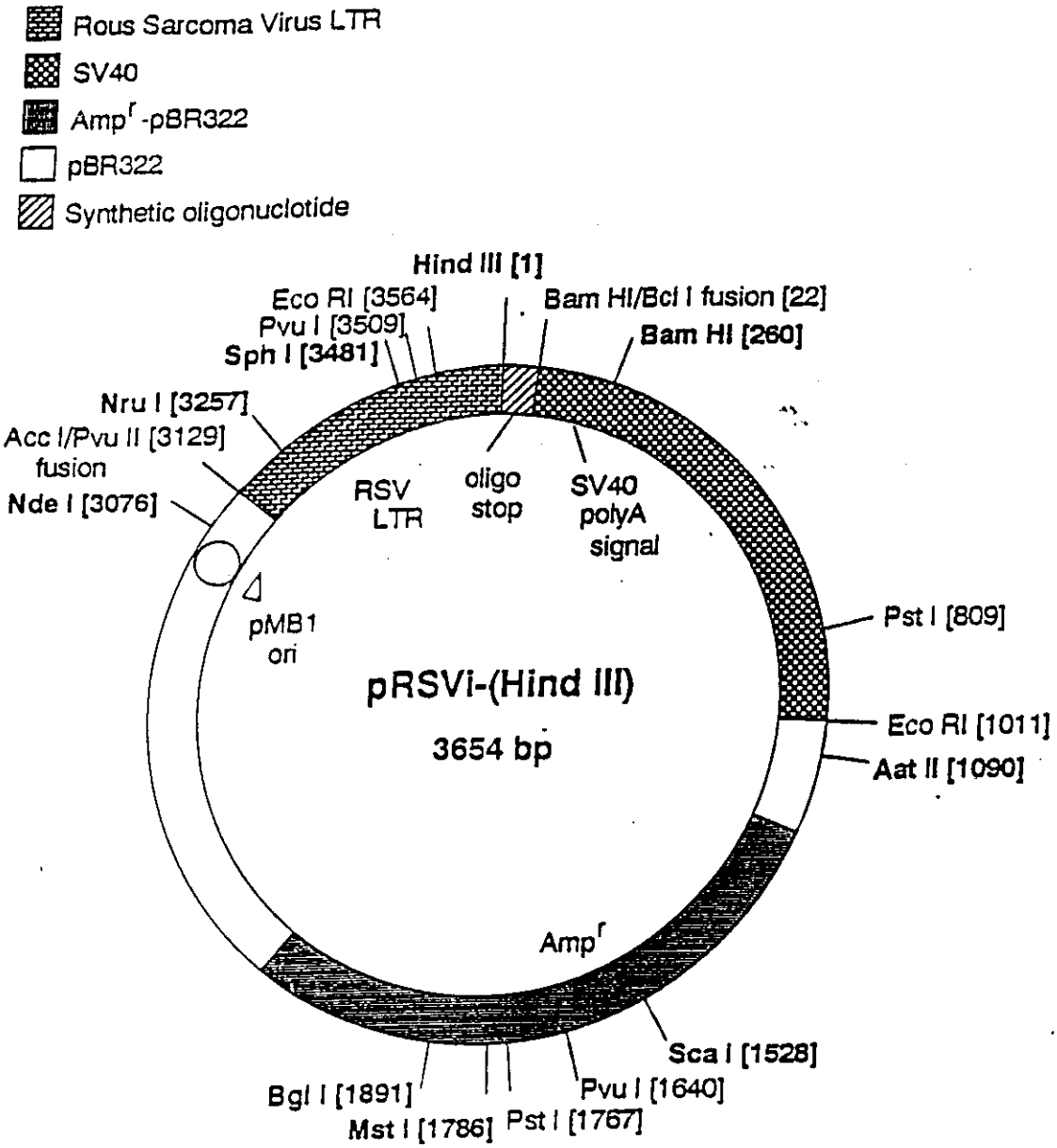


Hind III — Bam HI/Bcl I fusion

oligo stop = AAGCTTGCTGATTGATTGACCGGATCA

pSUPER RNAi System™

VECTOR: pSUPER.basic
CATALOG#: VEC-PBS-0001/0002

Length: 3176 bp

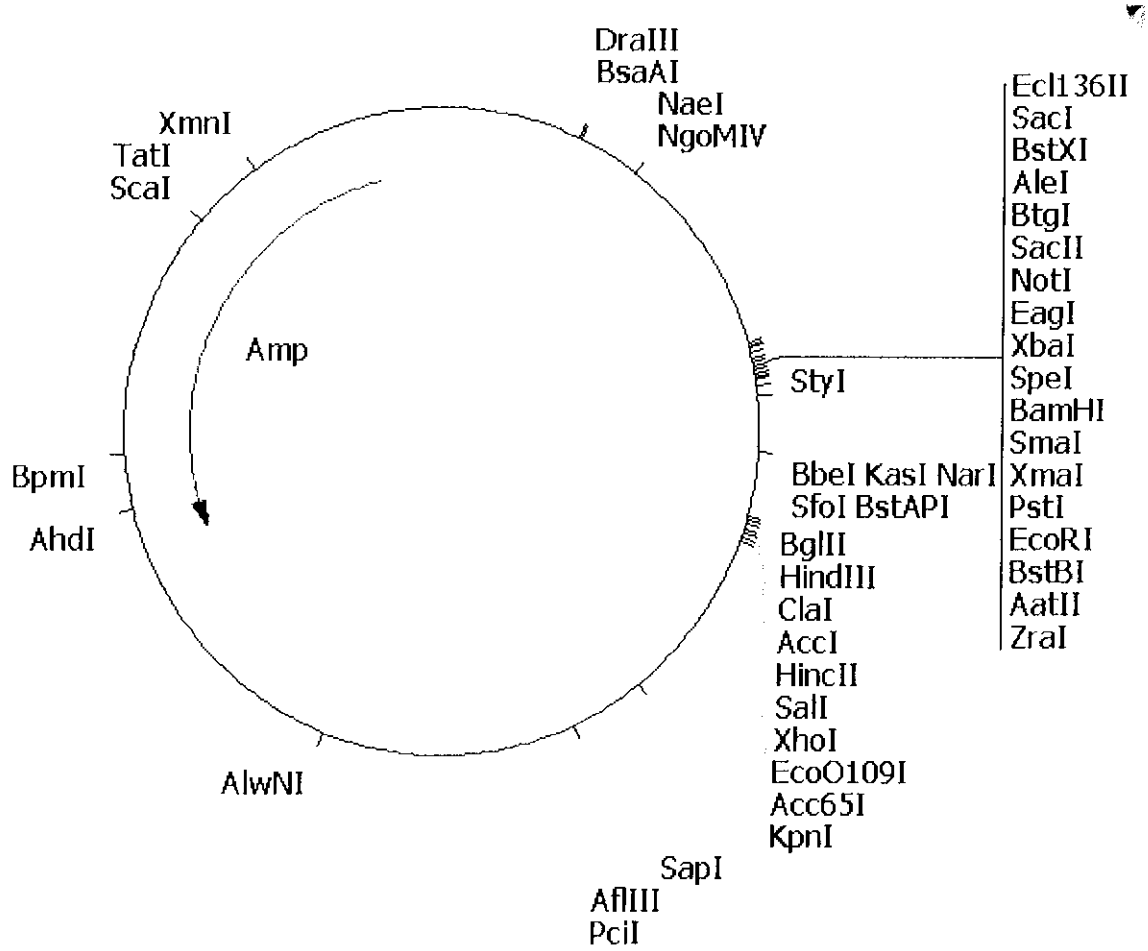
Key Sites

BglII: 928
HindIII: 934
EcoRI: 707
SalI: 949
XhoI: 955

Vector Features

f1(+) origin: 441-135
H1 promoter: 708 - 934
pUC origin: 1373-2040
Ampicillin resistance ORF: 3048-2191

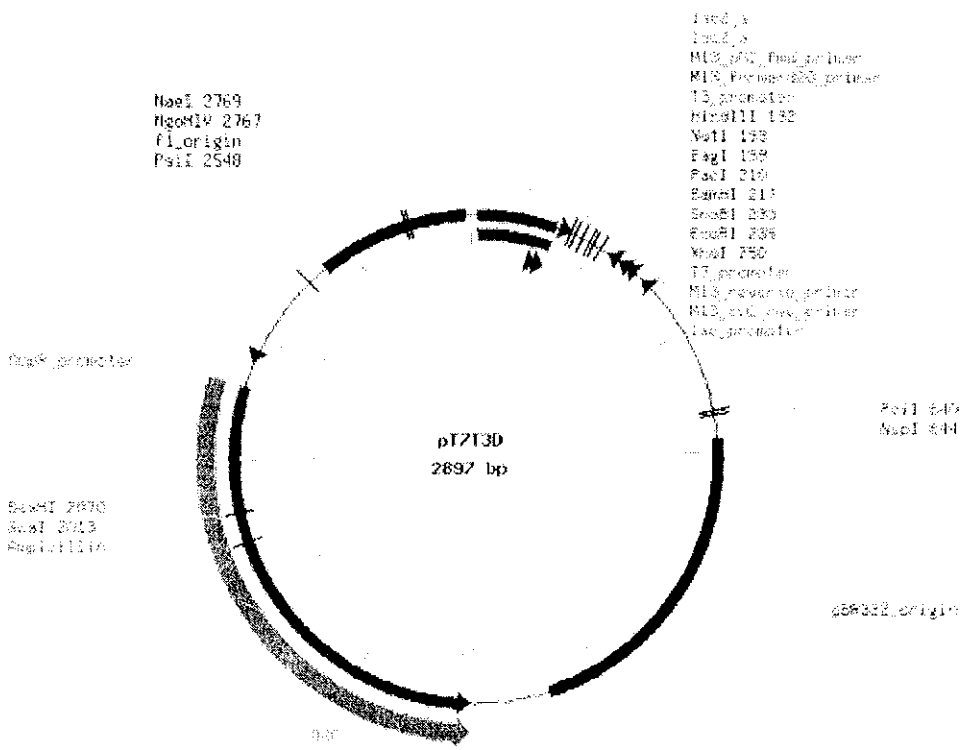
T7 primer binding site (AATACGACTCACTATAG): 627-643
T3 primer binding site (CTTTAGTGAGGGTTAAT): 989-1005
M13(-20) primer binding site (GTAAAACGACGGCCAGT): 600-616
M13 reverse primer binding site (CATGGTCATAGCTGTT): 1023-1038



addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pT7T3D
Alt Names	pT7T3D-PacI
Source/Vendor	Image Consortium
Promoter	T7
Plasmid Size	2897
Sequencing Primer	T7
Bacterial Resistance	Amp
Plasmid Sequence	View Sequence



Appendix A: Tet Vector Information

Clontech offers a wide variety of inducible expression vectors designed for use with Tet Expresssion Systems (Figure 8). Visit **www.clontech.com** for a complete list of currently available vectors. The vectors below are supplied with the Tet-Off Advanced Gene Expression System (Cat. No. 630934).

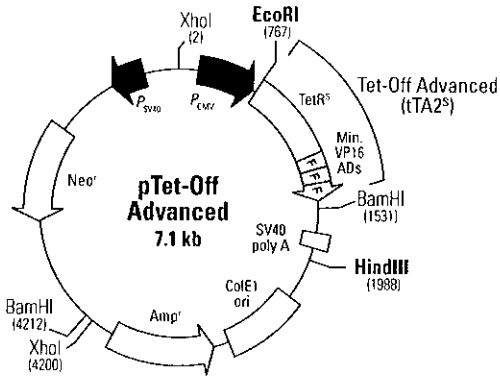


Figure 5. Map of pTet-Off Advanced. For a complete vector description, refer to the enclosed pTet-Off Advanced Vector Information Packet (PT3845-5).

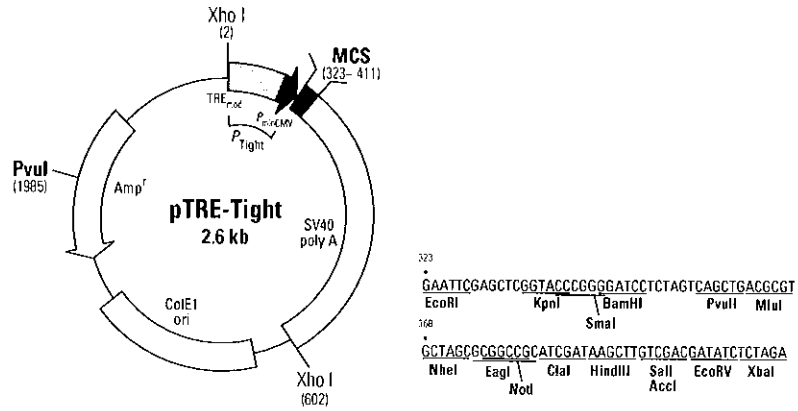


Figure 6. Map and MCS of pTRE-Tight. For a complete vector description, refer to the enclosed Vector Information Packet (PT3720-5).

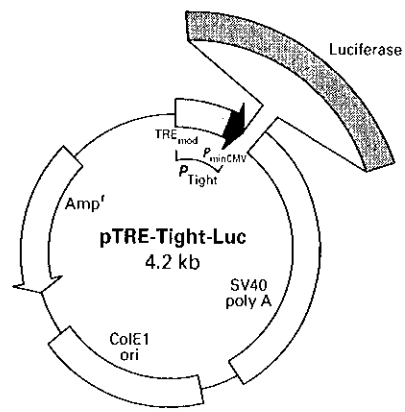


Figure 7. Map of pTRE-Tight-Luc.

Ambion®
THE RNA COMPANY®

pTRIamp19 Vector

NOTE: Commercial use of this vector requires a license from Ambion.

Catalog #: 7406 – 10 µg

Concentration: 0.5 mg/ml

Storage Conditions: Store at –20°C or 4°C.

All Ambion plasmids are shipped at ambient temperature for convenience and cost. This in no way affects the high quality performance of this plasmid. Upon receipt, store the plasmid at 4°C for frequent use or –20°C for long term storage.

Storage Buffer: 10 mM Tris-HCl pH 7.5, 1 mM EDTA.

Quality Control: The sequence of the polylinker and promoter regions of pTRI-amp-19 has been confirmed by dideoxynucleotide sequencing.

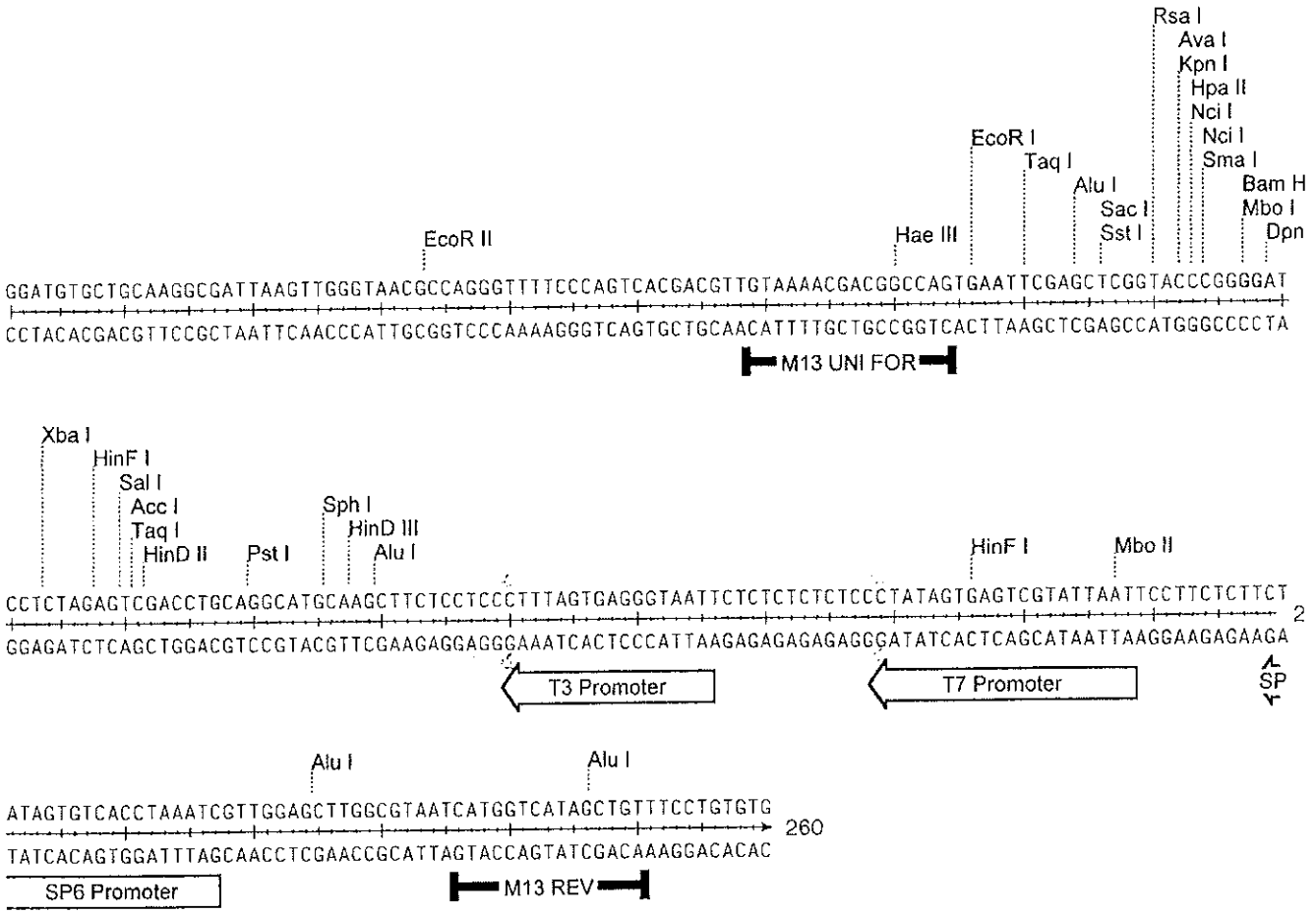
The vector is linearized with *Eco*R I, *Hin*d III and *Sma* I for 1 hour, re-ligated at room temperature for 3 hours, then tested for transformation, blue/white color selection and antibiotic resistance.

USER INFORMATION

pTRIamp19 is a triple tandem phage promoter transcription vector available exclusively from Ambion. When a DNA insert is cloned into the multiple cloning site and cut with a restriction enzyme on the distal side of the insert, such as *Eco* RI, the construct may be transcribed with any of the three phage RNA polymerases to make a transcript. Despite the close proximity to two other phage promoter sites, we have found no transcription from the inappropriate promoter, otherwise known as "cross-talk". All sites shown in the multiple cloning site are unique. pTRIamp19 was constructed using the vector pUC19 as a backbone and is 2782 bp long. This vector is ampicillin resistant and contains the β-galactosidase gene, thereby allowing blue-white color selection of transformants. Selection for the ampicillin marker is readily accomplished using a final concentration of 50 µg/ml ampicillin.

Each of the phage promoters in this vector is a CU Minus™ promoter, enabling the transcription of full length, high-specific activity RNA probes when the radiolabeled nucleotide is limited to as low as 0.3 µM.

Ambion, Inc. • 2130 WOODWARD STREET • AUSTIN, TEXAS 78744-1832 • TEL: (512)651-0200 • TOLL-FREE: (800)888-8804 • FAX: (512)651-0201 • WWW.AMBION.COM


Rsa I
Ava I
Kpn I
Hpa II
Nci I
Nci I
Sma I
Bam H
Mbo I
Dpn
EcoR I
Taq I
Alu I
Sac I
Sst I
Hae III
EcoR II
GGATGTGCTGCAAGGCGATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACGTTGTAAAACGACGGCCAGTGAATTCGAGCTCGGTACCCGGGGAT
CCTACACGACGTTCCGCTAATTCAACCCATTGCGGTCCCAAAAGGGTCAGTGCTGCAACATTTTGCTGCCGGTCACTTAAGCTCGAGCCATGGGCCCCTA
M13 UNI FOR
Xba I
HinF I
Sal I
Acc I
Taq I
HinD II
Pst I
Sph I
HinD III
Alu I
HinF I
Mbo II
CCTCTAGAGTCGACCTGCAGGCATGCAAGCTTCTCCTCCCTTTAGTGAGGGTAATTCTCTCTCTCTCCCTATAGTGAGTCGTATTAATTCCTTCTCTTCT
2
GGAGATCTCAGCTGGACGTCCGTACGTTCGAAGAGGAGGGAAATCACTCCCATTAAGAGAGAGAGAGGGATATCACTCAGCATAATTAAGGAAGAGAAGA
T3 Promoter
T7 Promoter
SP
Alu I
Alu I
ATAGTGTCACCTAAATCGTTGGAGCTTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTG
260
TATCACAGTGGATTTAGCAACCTCGAACCGCATTAGTACCAGTATCGACAAAGGACACAC
SP6 Promoter
M13 REV

Subject: Fwd: RE: Fwd: Biological Agents Registry Form: Strong
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Fri, 27 Aug 2010 13:32:57 -0400
To: kvolkening@robarts.ca, "'rsn@uwo.ca'" <rsn@uwo.ca>

Thanks Kathy
I understand from Ron that Dr. Dekaban has also reviewed this - so the official paperwork & approval will come through sometime next week,
Jennifer

-------- Original Message --------
Subject:RE: Fwd: Biological Agents Registry Form: Strong
Date: Fri, 27 Aug 2010 11:40:07 -0400
From: Kathryn Volkening <kvolkening@robarts.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: Ron Noseworthy <rnoseworthy@robarts.ca>, "lantz@robarts.ca" <lantz@robarts.ca>, Michael Strong <Michael.Strong@schulich.uwo.ca>

Hi Jennifer,

Please see the attached word document outlining the details asked for about the cDNA that we have used in our cloning in Dr. M.J. Strong's lab (to be included in the BARF submission). Anything else, please let me know by email (I will not be available in person between 10:30am Monday August 30 and 9am Tuesday September 7).
Thanks.

Kathy

From: Jennifer Stanley [mailto:jstanle2@uwo.ca]
Sent: August-25-10 4:12 PM
To: Kathryn Volkening; 'rsn@uwo.ca'
Subject: Fwd: Fwd: Biological Agents Registry Form: Strong

Hi Ron
Here is the e-mail without the file attachment,
Jennifer

-------- Original Message --------
Subject: Fwd: Biological Agents Registry Form: Strong
Date: Wed, 25 Aug 2010 14:44:19 -0400
From: Jennifer Stanley <jstanle2@uwo.ca>
To:kvolkening@robarts.ca
CC:'rsn@uwo.ca' <rsn@uwo.ca>

cDNA cloned in Dr. Michael Strong's Lab

There are no full genes (aka straight from the genome of any species, including introns and exons) cloned in this lab. All constructs have been created from cDNA (derived from RNA) from tissues/cells, and do not contain any of their native transcriptional regulatory elements (enhancers/repressors). All are expressed solely through regulatory elements contained in the commercial constructs (list provided in Appendix B. None of the cDNAs cloned have any known oncogenicity. All clones are from human cDNA origin with the exception of NF, TDP-43, FUS/TLS which have both human and mouse cDNA origin clones and 14-3-3 of which one isoform is of bovine cDNA origin.

Tau (all isoforms): These isoforms are thought to be housekeeping proteins that are observed to form "tangles" and plaques in neurodegenerative diseases such as Alzheimers disease-probably as a product of the disease and not a cause of the disease per se. These proteins have no known oncogenic properties.

RGNEF (all isoforms): RGNEF is a Rho Guanine Nucleotide Exchange Factor that is required for signaling within living cells. This protein serves to exchange GTP/GDP at the site of protein phosphorylation events, allowing for the propogation of signaling from the cell surface to target proteins within the cell. This is a housekeeper protein with no known oncogenic properties.

NFs (all forms, NFL, NFM and NFH; mouse and human): The neurofilaments are proteins that are integral to the structure of neurons. They form the "skeleton" of the neuron, and help to maintain cell shape. As well, neurofilament pathways within cells act as intracellular "highways" down which trafficking of other proteins, RNA and organelles occurs. These are ubiquitous proteins with no known oncogenic properties.

TDP-43 (all isoforms/truncation and mutations): TDP-43 is a dual RNA/DNA binding protein that is ubiquitously expressed and functions in the production of microRNA (small cellular RNA that regulate many cell functions), mRNA stability, mRNA export and trafficking throughout the cell. This protein has no known oncogenic properties. This protein appears to be overexpressed in some neurodegenerative diseases and in response to neuronal injury. Without this protein it appears cells cannot survive.

FUS/TLS (all isoforms/truncations and mutations): FUS/TLS is also a dual RNA/DNA binding protein that is ubiquitously expressed and functions in much the same manner as TDP-43. FUS/TLS has been described to form a fusion protein in liposarcoma, however, in the absense of a cancer state this protein does not form any fusions, and is not oncogenic.

14-3-3 (all isoforms): These proteins are ubiquitously expressed housekeepers. They serve as scaffolding proteins to facilitate the interaction of other proteins. They have no oncogenic properties.

Ubiquitin: Ubiquitin is a protein that is added by ubiquitin ligase to other proteins that have been targeted for degradation. This protein has no oncogenic properties.

Superoxide dismutase 1 (SOD1): SOD1 is a protein found predominantly in the mitochondria of mammalian cells. It functions as a free radical scavenger. It has no known oncogenic properties.

Vimentin: Vimentin is a cell scaffolding and cytoskeletal protein which functions with NFs to control cell shape, locomotion and cell-cell interfaces. It is not oncogenic.

Nestin: (See NF and Vimentin) Nestin is a cell cytoskeletal protein with the same functions as the NFs and Vimentin. It is not oncogenic.

Lamin: Lamin is a nuclear membrane located protein that functions in the maintanence of nuclear pores and the structure of the nuclear membrane. Nuclear pores are vital to the movement of materials like rRNA, miRNA, mRNA and various cell signaling proteins in and out of the nucleus. It is not oncogenic.

Progesterone receptor A and B (small fragments only 200 bases in size only, not the full receptor): The FULL receptor for progesterone has been linked with an increase in the proliferative capabilities of cancers. We have only 2 small 200 base fragments of these receptors cloned for in situ hybridization studies. These fragments are not oncogenic. The full receptors themselves serve to relay signals from the hormone progesterone to cells. The receptors themselves are not oncogenic. Please note that we have not cloned the entire receptors, simply 200 bases of each of them.