

# Modification Form for Permit BIO-RRI-0020

Permit Holder: *Joaquin Madrenas*

## Approved Personnel

(Please stroke out any personnel to be removed)

~~Gaitlin Lemke~~  
Thu Chau  
~~Mark Kirchoff~~  
Luan Chau  
~~Brianne Davis~~  
~~Sara Ramos~~  
Samar Sayedyahossein  
Darah Christie

## Additional Personnel

(Please list additional personnel here)

### Approved Microorganisms

Please stroke out any approved Biohazards to be removed below

~~Superantigen~~

Write additional Biohazards for approval below. \*

### Approved Cells

~~Human (established) - E. 6.1 JurKats, HEK 293 E, Rodent (primary, established), (Primary) - (Human): blood, cell lines. (Rodent) - Mice~~

### Approved Use of Human Source Material

~~blood, h. CTLA4 pbig2i Plasmid. Peritoneal fluid~~

human SLP-2-gfp cDNA in pBIG2i plasmid

### Approved GMO

~~E. Coli DH5 Alpha. (Plasmid) - pBIG2i~~

### Approved use of Animals

~~2007-078-12 for MICE~~

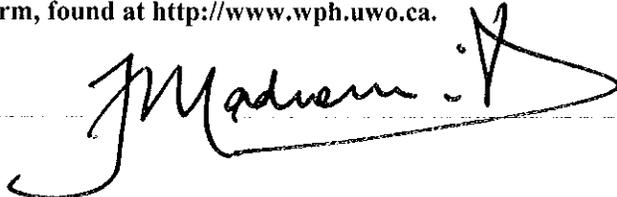
### Approved Toxin(s)

~~superantigen, SEE (Staphylococcal Enterotoxin E)~~

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

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

Signature of Permit Holder: \_\_\_\_\_



Classification: 2

Date of Last Biohazardous Agents Registry Form: Sep 23, 2009

Date of Last Modification (if applicable): \_\_\_\_\_

BioSafety Officer(s): \_\_\_\_\_

Chair, Biohazards Subcommittee: \_\_\_\_\_

**USE OF THIS NEW REAGENT:**

The SLP-2 protein was identified in a proteomics screening of lipid rafts of activated Jurkat T cells.

The cDNA was generated from these cells and subcloned in pBIG2i with a 3' gfp fusion.

See nucleotide and amino acid sequences attached.

We are currently using this construct to learn about the function of SLP-2.

Specific applications of this reagent are the transfection of the cDNA into the standard cell lines available in the laboratory, the induction of expression of a chimeric SLP-2-gfp protein, and the biochemical characterization of molecular partners for this protein using standard protein biochemistry techniques.

These experiments are within the standard operating procedures of a level 2 lab.

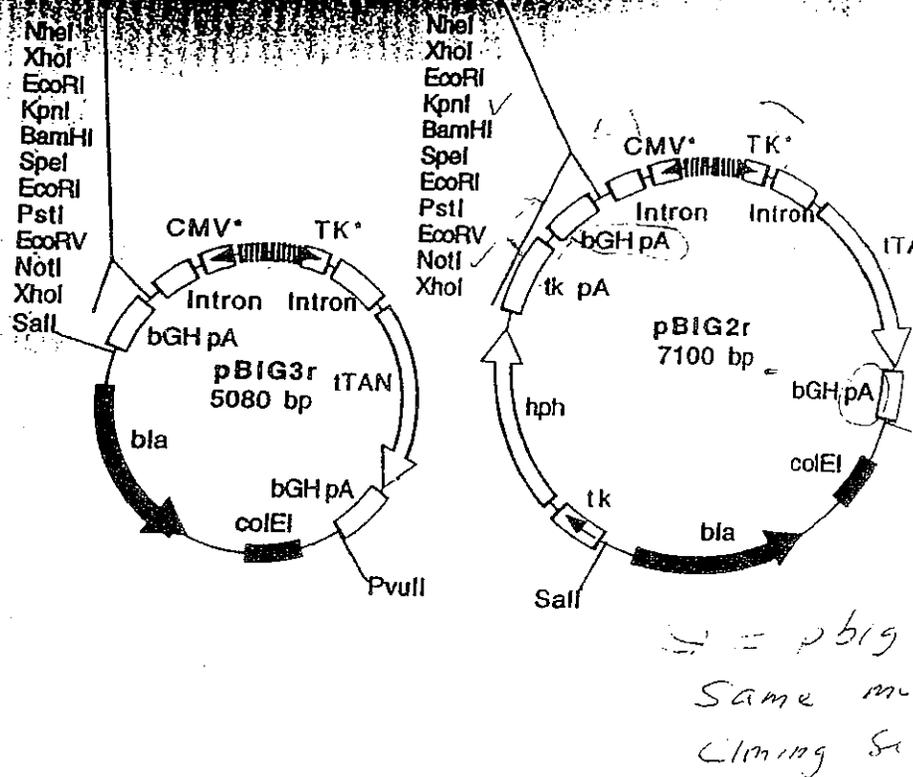


Fig.2: Autoregulated bi-directional tetracycline-responsive pBIG expression vectors. Each vector is based on a high copy number plasmid backbone containing the colE1 origin of replication and B<sup>l</sup>-lactamase gene that confers resistance to ampicillin. The bi-directional tetracycline-responsive promoter in each vector is comprised of a central tetO element, a stronger CMV\* element to drive cDNA expression and a weaker TK\* element to drive expression of the transactivator component. The two vectors are essentially identical with the exception that pBIG2 contains a selectable marker conferring resistance to hygromycin B for the generation of stable cell lines. The "i" series of vectors utilize the rtTAN transactivator such that cDNA expression is effectively induced by doxycycline.

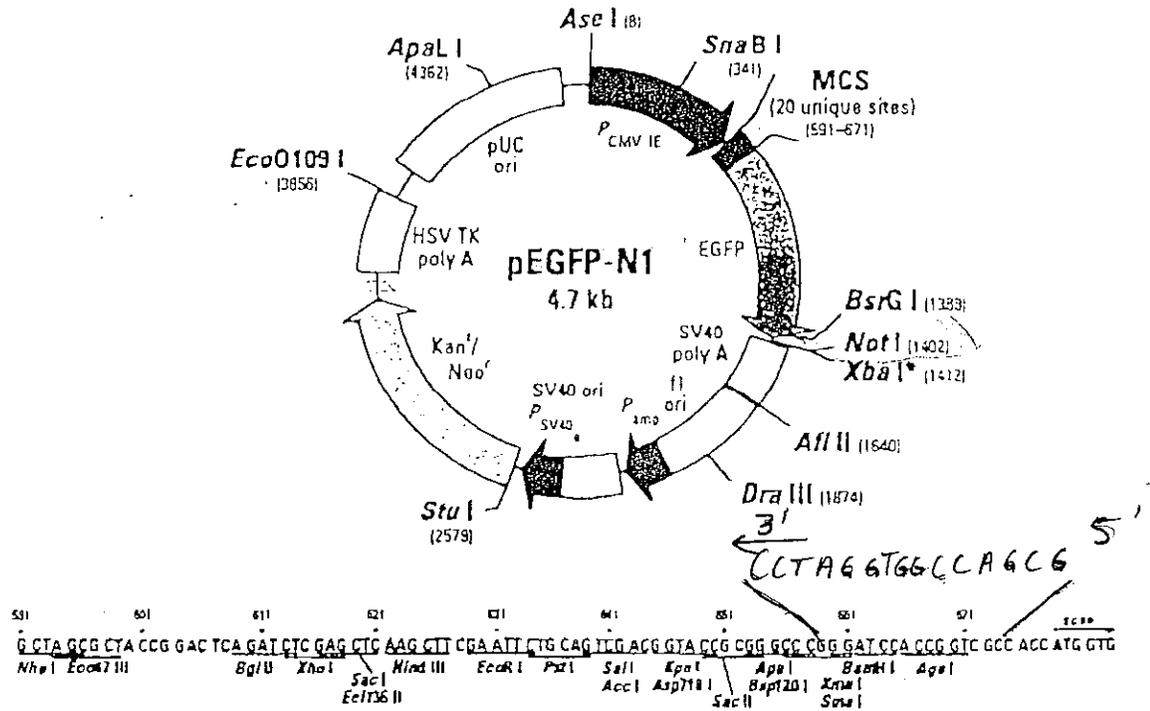
VECTOR INFORMATION

PT3027-5

pEGFP-N1 N-Terminal-Protein Fusion Vector

Catalog #6085-1

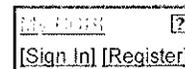
GenBank Accession #: U55762



**Restriction Map and Multiple Cloning Site (MCS) of pEGFP-N1.** (Unique restriction sites are in bold). The *Not*I site follows the EGFP stop codon. The *Xba*I site (\*) 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*<sup>-</sup> host 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 maxima = 488 nm; emission maxima = 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 ( $P_{CMVIE}$ ) and the EGFP coding sequences. Genes cloned into the MCS will be expressed as fusions to the N-terminus of EGFP if they are in the same reading frame as EGFP and there are no intervening stop codons. SV40 polyadenylation signals downstream of the EGFP gene direct proper processing of the 3' end of the EGFP mRNA. The vector backbone also contains an SV40 origin for replication in mammalian cells expressing the SV40 T antigen. A neomycin-resistance cassette (*neo*<sup>r</sup>), consisting of the SV40 early promoter, the neomycin/kanamycin resistance gene of Tn5, and polyadenylation signals from the Herpes simplex thymidine kinase gene, allows stably transfected eukaryotic cells to be selected using G418. A bacterial promoter upstream of this cassette ( $P_{amp}$ ) expresses kanamycin resistance in *E. coli*. The pGFP-N1 backbone also provides a pUC19 origin of replication for propagation in *E. coli* and an f1 origin for single-stranded DNA production.

Search  for

Go

Clear

[Limits](#) [Preview/Index](#) [History](#) [Clipboard](#) [Details](#)Format: [GenBank](#) [FASTA](#) [Graphics](#) [More Formats](#) ▼[Download](#) ▼[Save](#) ▼[Links](#) ▼

NCBI Reference Sequence: NM\_013442.1

## Homo sapiens stomatin (EPB72)-like 2 (STOML2), mRNA

[Change Region Shown](#)[Comment](#) [Features](#) [Sequence](#)[Customize View](#)

LOCUS NM\_013442 1303 bp mRNA linear PRI  
 01-NOV-2009  
 DEFINITION Homo sapiens stomatin (EPB72)-like 2 (STOML2), mRNA.  
 ACCESSION NM\_013442  
 VERSION NM\_013442.1 GI:7305502  
 KEYWORDS .  
 SOURCE Homo sapiens (human)  
 ORGANISM [Homo sapiens](#)  
 Euteleostomi;  
 Mammalia; Eutheria; Euarchontoglires; Primates;  
 Haplorrhini;  
 Catarrhini; Hominidae; Homo.  
 REFERENCE 1 (bases 1 to 1303)  
 AUTHORS Grass,S., Preuss,K.D., Ahlgrim,M., Fadle,N., Regitz,E.,  
 Pfoehler,C., Murawski,N. and Pfreundschuh,M.  
 TITLE Association of a dominantly inherited hyperphosphorylated  
 paraprotein target with sporadic and familial multiple  
 myeloma and  
 monoclonal gammopathy of undetermined significance: a  
 case-control  
 study  
 JOURNAL Lancet Oncol. 10 (10), 950-956 (2009)  
 PUBMED [19767238](#)  
 REMARK GeneRIF: Familial MGUS and multiple myeloma were  
 associated with a  
 dominant inheritance of hyperphosphorylated paratarg-7  
 REFERENCE 2 (bases 1 to 1303)  
 AUTHORS Kirchhof,M.G., Chau,L.A., Lemke,C.D., Vardhana,S.,  
 Darlington,P.J.,  
 Marquez,M.E., Taylor,R., Rizkalla,K., Blanca,I.,  
 Dustin,M.L. and  
 Madrenas,J.  
 TITLE Modulation of T cell activation by stomatin-like protein  
 2  
 JOURNAL J. Immunol. 181 (3), 1927-1936 (2008)  
 PUBMED [18641330](#)  
 REMARK GeneRIF: SLP-2 is an important player in T cell  
 activation by  
 ensuring sustained TCR signaling  
 REFERENCE 3 (bases 1 to 1303)  
 AUTHORS Green,J.B. and Young,J.P.  
 TITLE Slipins: ancient origin, duplication and diversification  
 of the  
 stomatin protein family  
 JOURNAL BMC Evol. Biol. 8, 44 (2008)  
 PUBMED [18267007](#)  
 REMARK GeneRIF: Endosymbiotic origin of paraslipin from an  
 alphaproteobacterial ancestor (SLP-2)  
 Publication Status: Online-Only  
 REFERENCE 4 (bases 1 to 1303)  
 AUTHORS Cao,W., Zhang,B., Liu,Y., Li,H., Zhang,S., Fu,L., Niu,Y.,

[Analyze This Sequence](#)[Run BLAST](#)[Pick Primers](#)[Articles about the STOML2 gene](#)[Association of a dominantly inherited hyp](#)[Lancet Oncol. 2009][Modulation of T cell activation by stomatin-like pr](#)[J Immunol. 2008][Slipins: ancient origin, duplication and diversif](#)[BMC Evol Biol. 2008]

» See all...

[RefSeq Protein Product](#)

See the reference protein sequence for stomatin (EPB72)-like 2 (NP\_038470.1).

[More about the STOML2 gene](#)

Also Known As: HSPC108, SLP-2

[Homologs of the STOML2 gene](#)

The STOML2 gene is conserved in chimpanzee, dog, cow, mouse, rat, zebrafish, fruit fly, mosquito, C.elegans, S.pombe, M.grisea, N.crassa, A.thaliana, rice, and P.falciplarum.

[Recent activity](#)[Turn Off](#) [Clear](#)

Homo sapiens stomatin (EPB72)-like 2 (STOML2),

STOML2 stomatin (EPB72)-like 2 (Homo

[human SLP-2 \(11\)](#) Protein

» See more...

[All links from this record](#)

<p>Ning,L.,          Cao,X., Liu,Z. and Sun,B.  <b>TITLE</b> High-level SLP-2 expression and HER-2/neu protein          expression are          associated with decreased breast cancer patient survival  <b>JOURNAL</b> Am. J. Clin. Pathol. 128 (3), 430-436 (2007)  <b>PUBMED</b> <a href="#">17709317</a>  <b>REMARK</b> GeneRIF: High-level SLP-2 expression was associated with          decreased          patients          overall survival (P = .011) and was more often found in          advanced          with tumors larger than 20 mm, lymph node metastasis,          clinical stage, distant metastasis  <b>REFERENCE</b> 5 (bases 1 to 1303)  <b>AUTHORS</b> Cui,Z., Zhang,L., Hua,Z., Cao,W., Feng,W. and Liu,Z.  <b>TITLE</b> Stomatin-like protein 2 is overexpressed and related to          cell growth          in human endometrial adenocarcinoma  <b>JOURNAL</b> Oncol. Rep. 17 (4), 829-833 (2007)  <b>PUBMED</b> <a href="#">17342323</a>  <b>REMARK</b> GeneRIF: SLP-2 was overexpressed in endometrial          adenocarcinoma          compared with their normal counterparts.  <b>REFERENCE</b> 6 (bases 1 to 1303)  <b>AUTHORS</b> Guo,D., Han,J., Adam,B.L., Colburn,N.H., Wang,M.H.,          Dong,Z.,          Eizirik,D.L., She,J.X. and Wang,C.Y.  <b>TITLE</b> Proteomic analysis of SUMO4 substrates in HEK293 cells          under serum          starvation-induced stress  <b>JOURNAL</b> Biochem. Biophys. Res. Commun. 337 (4), 1308-1318 (2005)  <b>PUBMED</b> <a href="#">16236267</a>  <b>REFERENCE</b> 7 (bases 1 to 1303)  <b>AUTHORS</b> Rush,J., Moritz,A., Lee,K.A., Guo,A., Goss,V.L.,          Spek,E.J.,          Zhang,H., Zha,X.M., Polakiewicz,R.D. and Comb,M.J.  <b>TITLE</b> Immunoaffinity profiling of tyrosine phosphorylation in cancer          cells  <b>JOURNAL</b> Nat. Biotechnol. 23 (1), 94-101 (2005)  <b>PUBMED</b> <a href="#">15592455</a>  <b>REFERENCE</b> 8 (bases 1 to 1303)  <b>AUTHORS</b> Humphray,S.J., Oliver,K., Hunt,A.R., Plumb,R.W., Loveland,J.E.,          Howe,K.L., Andrews,T.D., Searle,S., Hunt,S.E., Scott,C.E.,          Jones,M.C., Ainscough,R., Almeida,J.P., Ambrose,K.D., Ashwell,R.I.,          Babbage,A.K., Babbage,S., Bagguley,C.L., Bailey,J., Banerjee,R.,          Barker,D.J., Barlow,K.F., Bates,K., Beasley,H., Beasley,O.,          Bird,C.P., Bray-Allen,S., Brown,A.J., Brown,J.Y., Burford,D.,          Burrill,W., Burton,J., Carder,C., Carter,N.P., Chapman,J.C.,          Chen,Y., Clarke,G., Clark,S.Y., Clee,C.M., Clegg,S., Collier,R.E.,          Corby,N., Crosier,M., Cummings,A.T., Davies,J., Dhama,P., Dunn,M.,          Dutta,I., Dyer,L.W., Earthrowl,M.E., Faulkner,L., Fleming,C.J.,          Frankish,A., Frankland,J.A., French,L., Fricker,D.G., Garner,P.,          Garnett,J., Ghorri,J., Gilbert,J.G., Glison,C., Grafham,D.V.,          Gribble,S., Griffiths,C., Griffiths-Jones,S., Grocock,R., Guy,J.,          Hall,R.E., Hammond,S., Harley,J.L., Harrison,E.S., Hart,E.A.,          Heath,P.D., Henderson,C.D., Hopkins,B.L., Howard,P.J., Howden,P.J.,          Huckle,E., Johnson,C., Johnson,D., Joy,A.A., Kay,M., Keenan,S.,          Kershaw,J.K., Kimberley,A.M., King,A., Knights,A., Laird,G.K.,          Langford,C., Lawlor,S., Leongamornlert,D.A., Leversha,M., Lloyd,C.,          Lloyd,D.M., Lovell,J., Martin,S., Mashreghi-Mohammadi,M.,          Matthews,L., McLaren,S., McLay,K.E., McMurray,A., Milne,S.,          Nickerson,T., Nisbett,J., Nordsiek,G., Pearce,A.V., Peck,A.I.,          Porter,K.M., Pandian,R., Pelan,S., Phillimore,B., Povey,S.,          Ramsey,Y., Rand,V., Scharfe,M., Sehra,H.K., Shownkeen,R.,          Sims,S.K., Suke,C.D., Smith,M., Steward,C.A., Swarbreck,D.,          Sycamore,N., Tester,J., Thorpe,A., Tracey,A., Tromans,A.,          Thomas,D.W., Wall,M., Wallis,J.M., West,A.P., Whitehead,S.L.,          Willey,D.L., Williams,S.A., Wilming,L., Wray,P.W., Young,L.,</p>	<p>Related sequences          Full text in PMC          GEO profiles          Gene          Gene genotype          GeneView in dbSNP          Genome          HomoloGene          Map viewer          Master          OMIM          Order cDNA clone          Probe          Protein          PubMed          PubMed (RefSeq)          PubMed (weighted)          SNP          Taxonomy          UniGene          UniSTS          mRNA genome project          LinkOut</p>
--	--

Ashurst,J.L., Coulson,A., Blocker,H., Durbin,R., Sulston,J.E.,  
Hubbard,T., Jackson,M.J., Bentley,D.R., Beck,S., Rogers,J. and  
Dunham,I.

TITLE DNA sequence and analysis of human chromosome 9  
JOURNAL Nature 429 (6990), 369-374 (2004)  
PUBMED [15164053](#)  
REFERENCE 9 (bases 1 to 1303)  
AUTHORS Owczarek,C.M., Treutlein,H.R., Portbury,K.J., Gulluyan,L.M.,  
Kola,I. and Hertzog,P.J.  
TITLE A novel member of the STOMATIN/EPB72/mec-2 family, stomatin-like 2  
(STOML2), is ubiquitously expressed and localizes to HSA chromosome  
9p13.1  
JOURNAL Cytogenet. Cell Genet. 92 (3-4), 196-203 (2001)  
PUBMED [11435687](#)  
REFERENCE 10 (bases 1 to 1303)  
AUTHORS Wang,Y. and Morrow,J.S.  
TITLE Identification and characterization of human SLP-2, a novel  
homologue of stomatin (band 7.2b) present in erythrocytes and other  
tissues  
JOURNAL J. Biol. Chem. 275 (11), 8062-8071 (2000)  
PUBMED [10713127](#)  
COMMENT PROVISIONAL REFSEQ: This record has not yet been subject to final  
NCBI review. The reference sequence was derived from [AF190167.1](#).

Publication Note: This RefSeq record includes a subset of the  
publications that are available for this gene. Please see the  
Entrez Gene record to access additional publications.

FEATURES Location/Qualifiers  
source 1..1303  
/organism="Homo sapiens"  
/mol\_type="mRNA"  
/db\_xref="taxon:9606"  
/chromosome="9"  
/map="9p13.1"  
gene 1..1303  
/gene="STOML2"  
/gene\_synonym="HSPC108; SLP-2"  
/note="stomatin (EPB72)-like 2"  
/db\_xref="GeneID:30968"  
/db\_xref="HGNC:14559"  
/db\_xref="HPRD:16310"  
/db\_xref="MIM:608292"  
exon 1..108  
/gene="STOML2"  
/gene\_synonym="HSPC108; SLP-2"  
/inference="alignment:Splign"  
/number=1  
CDS 64..1134  
/gene="STOML2"  
/gene\_synonym="HSPC108; SLP-2"  
/note="stomatin-like protein 2; stomatin-like 2"  
/codon\_start=1  
/product="stomatin (EPB72)-like 2"  
/protein\_id="NP\_038470.1"  
/db\_xref="GI:7305503"  
/db\_xref="CCDS:CCDS6577.1"  
/db\_xref="GeneID:30968"  
/db\_xref="HGNC:14559"  
/db\_xref="HPRD:16310"  
/db\_xref="MIM:608292"  
/translation="MLARAARGTGALLRGSLLASGRAPRRASSGLPRNTVVLFVPPQQ  
EAWVVERMGRFHRILEPGLNIIIPVLDRIYVQSLKEIVINVPEQSAVTLDNVTLQID  
GVLYLRIMDPYKASYGVDEPEYAVTQLAQTTRSELGKLSLDKVFRERESLNASIVDA  
INQAADCWGIKRLRYEIKDIHVPPRVKESMQMVEAERRKRATVLESEGTRESAINVA  
EGKKQAQILASEAEKAEQINQAAGEASAVLAKAKAKAEAIRILAAALTQHNGDAASL  
TVAEQYVSFAFKLAKDSNTILLPSNPGDVTSMVAQAMGVYGALTKAPVPGTPDSLSSG  
SSRDVQGTDAASLDEELDRVKMS"  
exon 109..246  
/gene="STOML2"

```

        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=2
    exon      247..346
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=3
    exon      347..405
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=4
    exon      406..507
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=5
    exon      508..642
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=6
    exon      643..787
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=7
    exon      788..867
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=8
    exon      868..996
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=9
    exon      997..1278
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /inference="alignment:Splign"
        /number=10
    STS      1041..1192
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /standard_name="A002P16"
        /db_xref="UniSTS:43594"
    STS      1069..1200
        /gene="STOML2"
        /gene_synonym="HSPC108; SLP-2"
        /standard_name="RH41785"
        /db_xref="UniSTS:12941"

```

## ORIGIN

```

1  ggcttctg  agcgaccgct  ccgctcgtct  cgttggtcc  ggaggtcgct  gcggcgggtg
61  gaaatgctg  cgcgcgcggc  gcggggcact  ggggcccttt  tgotgagggg  ctctctactg
121 gcttctgccc  gcgctccgcg  ccgcgcctcc  tctggattgc  cccgaaacac  cgtgggtactg
181 ttcgtgccgc  agcaggaggc  ctgggtggtg  gagcgaatgg  gccgattcca  ccggtacctg
241 gagcctggtt  tgaacatcct  catccctgtg  ttagaccgga  tccgatatgt  gcagagtctc
301 aaggaaattg  tcatcaacgt  gcctgagcag  tcggctgtga  ctctcgacaa  tgtaactctg
361 caaatcgatg  gagtccctta  cctgcgcata  atggaccctt  acaaggcaag  ctacgggtg
421 gaggaccctg  agtatgccgt  caccagccta  gctcaaacia  ccatgagatc  agagctcggc
481 aaactctctc  tggacaaaag  cttccgggaa  cgggagtccc  tgaatgccag  cattgtggat
541 gccatcaacc  aagctgctga  ctgctggggg  atccgctgcc  tccgttatga  gatcaaggat
601 atccatgtgc  caccocgggt  gaaagagtct  atgcagatgc  aggtggaggc  agagcggcgg
661 aaacgggcca  cagttctaga  gtctgagggg  acccgagagt  cggccatcaa  tgtggcagaa
721 ggaagaagaa  aggccagat  cctggcctcc  gaagcagaaa  aggctgaaca  gataaatcag
781 gcagcaggag  aggccagtgc  agttctggcg  aaggccaagg  ctaaagctga  agctattcga
841 atcctggctg  cagctctgac  acaacataat  ggagatgcag  cagcttact  gactgtggcc

```

```
901 gagcagtatg tcagcgcggt ctccaaactg gccaaaggact ccaaacactat cctactgccc
961 tccaaccctg gcgatgtcac cagcatggtg gctcaggcca tgggtgtata tggagcctc
1021 accaaagccc cagtgccagg gactccagac tcactctcca gtgggagcag cagagatgtc
1081 cagggtacag atgcaagtct tgatgaggaa cttgatcgag tcaagatgag ttagtgagc
1141 tgggcttggc cagggagtct ggggacaagg aagcagattt tcttgattct ggctctagct
1201 tccctgcaa gattttggtt tttattttt tatttgaact ttagtcgtgt aataaactca
1261 ccagtggcaa acctgaaaaa aaaaaaaaaa aaaaaaaaaa aaa
```

//

[Write to the Help Desk](#)  
[NCBI | NLM | NIH](#)  
[Department of Health & Human Services](#)  
[Privacy Statement](#) | [Freedom of Information Act](#) | [Disclaimer](#)

# Modification Form for Permit BIO-RRI-0020

Permit Holder: Joaquin Madrenas

## Approved Personnel

(Please stroke out any personnel to be removed)

~~Gaitlin-Lemke~~  
~~Mark-Kirchhof~~  
~~Luan Chau~~  
~~Brianne-Davis~~

## Additional Personnel

(Please list additional personnel here)

Thu Chau  
Darah Christie  
Samas Sayedyahosseini  
Isaac Elias

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms		
Approved Cells	Human (primary, established), Rodent (primary, established), Jurkat, Hek 293	
Approved Use of Human Source Material	blood	<u>h.CTLA4 pbig2i plasmid</u>
Approved GMO		
Approved use of Animals		

\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Oct 12, 2006

Signature of Permit Holder: Joaquin Madrenas

BioSafety Officer(s): W. Tanley June 24/09

Chair, Biohazards Subcommittee: G.M. Kessler

# Modification Form for Permit BIO-RRI-0020

Permit Holder: *Joaquin Madrenas*

Approved Toxin(s)

superantigen

\* The h.CTLA4 pbig2i plasmid is used to transfect human T cell lines (Jurkats) to express CTLA-4 under doxycycline regulation. The transfected cell lines are then used to test their responses in-vitro to different stimuli.

\* PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.

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

Classification: 2

Date of last Biohazardous Agents Registry Form: Oct 12, 2006

Signature of Permit Holder: *J. Madrenas*

BioSafety Officer(s): *J. Stanley* *June 26/09*

Chair, Biohazards Subcommittee: *S.M. Kelder*



**BIOHAZARDOUS AGENTS REGISTRY FORM**

Reviewed by Biosafety Subcommittee: February 2006

*This form must be completed by each Principal Investigator when completing a grant application or grant renewal to be administered by the Robarts Research Institute, if the use of biohazardous and/or infectious agents is proposed. For any proposed animal work involving the use of biohazardous agents or animals carrying zoonotic agents infectious to humans, this form must also be completed.*

**COMPLETED FORMS ARE TO BE RETURNED TO BIOSAFETY SUBCOMMITTEE CHAIR,  
ROOM 3-34.1.**

*If there are any changes to the information on these forms (excluding grant title and funding agencies) a new form must be completed and sent to the Biosafety Subcommittee Chair **BEFORE** implementation of these changes can occur.*

*If multi-team grants are being applied for, each individual investigator of the team must submit a Biohazardous Agents Registry Form to the Biosafety Subcommittee Chair.*

Containment Levels will be required in accordance with Health Canada (HC), Laboratory Biosafety Guidelines, 3<sup>rd</sup> edition 2004, or Canadian Food Inspection Agency (CFIA), Containment Standards for Veterinary Facilities, 1<sup>st</sup> edition 1996.

For questions regarding this form, please contact Biosafety Subcommittee Chair at ext. 34125.

**1.0 Contact Information**

PRINCIPAL INVESTIGATOR: Joaquin Madrenas

SIGNATURE: J Madrenas

DATE: Aug 23/06

DEPARTMENT: Microbiology + Immunology

ADDRESS: RRI

TELEPHONE: x 342-42

EMAIL: madrenas@robarts.ca

Location of experimental work to be carried out:

Building(s): RRI

Room(s): 2-05

*\*For work being performed at Institutions affiliated with the Robarts Research Institute, the Safety Officer for the Institution where experiments will take place must sign the form prior to it being sent to Robarts Research Institute, Biosafety Subcommittee Chair. See Section 13.0, Approvals*

GRANT TITLE(S): The role of SLP-2 in TCR signalosome assembly and T cell activation

ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH AS THE RESEARCH GRANT SUMMARY(S) EXPLAINING THE BIOHAZARD(S) USED.

FUNDING AGENCY/AGENCIES: CHRR NCIC

Anticipated Grant End Date: \_\_\_\_\_

Names of all personnel working under Principal Investigator's supervision in this location:

- Ms. LUAN A. CHAU \_\_\_\_\_
- Dr. CAIT D. LETIKE \_\_\_\_\_
- Dr. MARK G. KIRCHHOF \_\_\_\_\_
- Ms. BRIANNE DAVLS \_\_\_\_\_
- Ms THU A. CHAU \_\_\_\_\_
- \_\_\_\_\_
- \_\_\_\_\_

Note : A list of human pathogens categorized according to Risk Group can be obtained by calling the Office of Laboratory Security directly at (613) 957-1779 or accessing their Web site : <http://www.phac-aspc.gc.ca/ols-bsl/index.html>

**2.0 Microorganisms**

2.1 Does your work involve the use of microorganisms? YES  NO   
If NO, please proceed to Section 3.0

2.2 Please complete the table below:

Name of Microorganism	Is microorganism a known human pathogen? YES/NO	Is microorganism a known animal pathogen? YES/NO	Is microorganism a known zoonotic agent? YES/NO	Maximum quantity to be cultured at one time?	Health Canada or CFIA Containment Level (select one)
					1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>
					1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>
					1 <input type="radio"/> 2 <input type="radio"/> 3 <input type="radio"/>

**3.0 Cell Culture**

3.1 Does your work involve the use of cell cultures? YES  NO   
 If NO, please proceed to Section 4.0.

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

Cell Type	Is this cell type used in your work? YES / NO	Established or Primary *	Supplier of Primary Cell Culture Tissue
Human	yes	both	volunteers
Rodent	yes	both	mice
Non-human primate			
Other (specify)			

\* i.e. derived from fresh tissue

3.3 Complete the following table.

Specific Cell Line	Source / Supplier	HC or CFIA Containment Level (select one)		
JURKAT	ATCC	1 <input type="checkbox"/>	2 <input checked="" type="checkbox"/>	3 <input type="checkbox"/>
HEK293	ATCC	1 <input type="checkbox"/>	2 <input checked="" type="checkbox"/>	3 <input type="checkbox"/>
		1 <input type="checkbox"/>	2 <input type="checkbox"/>	3 <input type="checkbox"/>

**4.0 Use of Human Source Materials**

4.1 Does your work involve the use of human source materials? YES  NO   
 If NO, please proceed to Section 5.0

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

Human Source Material	Specify Source, or Not Applicable (NA)	Is Human Source Material known to be infected with an infectious agent? YES/NO	Name of Infectious Agent	HC or CFIA Containment Level (select one)
Human Blood (whole) or other Body Fluid	volunteer	No		1 <input type="checkbox"/> 2 <input checked="" type="checkbox"/> 3 <input type="checkbox"/>
Human Blood (fraction) or other Body Fluid				1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/>
Human Organs (unpreserved)				1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/>
Human Tissues (unpreserved)				1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/>

**5.0 Genetically Modified Organisms and Cell lines**

5.1 Will genetic modifications be made to the organism, virus or cell line? YES  NO   
If NO, please proceed to Section 6.0

5.2 Will genetic sequences from any of the following be involved?

- HIV YES  NO

If YES, specify: \_\_\_\_\_

- HTLV 1 or 2 YES  NO

If YES, specify: \_\_\_\_\_

- Other human or animal pathogen and/or their toxins YES  NO

If YES, specify: \_\_\_\_\_

5.2 Will intact genetic sequences be used from:

- SV 40 Large T antigen YES  NO
- Adeno E1A YES  NO
- Known or suspected oncogenes YES  NO

If YES, specify: \_\_\_\_\_

5.4 Will a live vector(s) (viral or bacterial) be used for gene transduction? YES  NO

If YES, name virus: \_\_\_\_\_

5.5 List specific vector(s) to be used: \_\_\_\_\_

5.6 Will virus be replication defective? YES  NO

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

5.8 Will this be expected to increase the Containment Level required? YES  NO

**6.0 Human Gene Therapy Trials**

6.1 Will human clinical trials using the viral vector in 4.0 be conducted? YES  NO   
If NO, please proceed to Section 7.0  
If YES, attach a full description of the make-up of the virus.

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

6.3 How will the virus be administered? \_\_\_\_\_

6.4 Please give the Health Care Facility where the clinical trial will be conducted:  
\_\_\_\_\_

6.5 Has human ethics approval been obtained? YES  NO

Approval # \_\_\_\_\_

**7.0 Animal Experiments**

- 7.1 Will any of the agents listed be used in live animals? YES  NO   
If NO, please proceed to section 8.0
- 7.2 Name of animal species to be used: \_\_\_\_\_
- 7.3 AUS protocol # \_\_\_\_\_
- 7.4 If using murine cell lines, have they been tested for murine pathogens? YES  NO

**8.0 Use of Animal species with Zoonotic Hazards**

- 8.1 Will any of the following animals or their organs, tissues, lavages or other bodily fluids including blood be used?
- Pound source dogs YES  NO
  - Pound source cats YES  NO
  - Sheep or goats YES  NO
  - Non- Human Primates YES  NO

If YES specify species \_\_\_\_\_

- Wild caught animals YES  NO

If YES specify species \_\_\_\_\_

**9.0 Biological Toxins**

- 9.1 Will toxins of biological origin be used? YES  NO   
If NO, please proceed to Section 10.0  
If YES, please name the toxin SUPER ANTIGEN.
- 9.2 What is the LD<sub>50</sub> (specify species) of the toxin? ≈ 2 PICOMOLES.

*which one?  
details storage  
source -  
enactment -  
how to be  
used ??  
since info  
not in  
summary.*

**10.0 Import Requirements**

- 10.1 Will the agent be imported? YES  NO   
If NO, please proceed to Section 11.0  
If YES, country of origin \_\_\_\_\_
- 10.2 Has an Import Permit been obtained from HC for human pathogens? YES  NO
- 10.3 Has an import permit been obtained from CFIA for animal pathogens? YES  NO
- 10.4 Has the import permit been sent to Biosafety Subcommittee Chair? YES  NO   
If YES, Permit # \_\_\_\_\_

**11.0 Training Requirements for Personnel Named on Form**

All personnel named in section 1.0 of this form who will be using any of the above named agents are required to attend the following training courses given by OH&S.

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 2.0 to 10.0 have been trained as required.

SIGNATURE J Madren

**12.0 Containment Levels**

12.1 For the work described in sections 2.0 to 10.0, select the highest HC or CFIA Containment Level required.                      10                      20                       30

12.2 Has the facility been certified by Biosafety Subcommittee Chair for this level of containment?  
YES                       NO

If YES, give date: NOV 25/2004 and permit number: P-10863  
March 15, 2005                      2005-03-(2105)

**13.0 Approvals**

Robarts Research Institute

Signature [Signature] Date Oct 12, 2005

Biosafety Officer for the institution where experiments will take place

Signature \_\_\_\_\_ Date \_\_\_\_\_

Biosafety Officer of Robarts Research Institute (if different than above)

Signature \_\_\_\_\_ Date \_\_\_\_\_

Note: This permit will be in effect from \_\_\_\_\_ to \_\_\_\_\_  
subject to annual facility re-certification.