

pSELECT-GFP-LC3

A mammalian expression plasmid containing the human LC3B gene fused at 5' end to the GFP gene

Catalog # psetz-gfplc3

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-GFP-LC3 plasmid provided as lyophilized DNA
- 3' UTR of SV40 late polyadenylation signal

Storage and Stability:

Product is shipped at room temperature. Lyophilized DNA should be resuspended upon receipt and stored at -20°C. Lyophilized DNA is stable for 3 months at -20°C. Resuspended DNA is stable more than one year at -20°C.

Store Zeocin™ at 4 °C or at -20 °C. The expiry date is specified on the product label.

Quality control:

Plasmid construct has been confirmed by restriction analysis and sequencing. Plasmid DNA was purified by ion exchange chromatography and lyophilized.

GENERAL PRODUCT USE

pSELECT plasmids are specifically designed for strong and constitutive expression of a gene of interest in a wide variety of cell lines. They allow the selection of stable transfectants and offer a variety of selectable markers. pSELECT plasmids contain two expression cassettes: the first drives the expression of the gene of interest and the second drives the expression of a large choice of dominant selectable markers for both *E. coli* and mammalian cells. They are both terminating with a strong polyadenylation signal (polyA) that separates the two expression cassettes thus preventing any transcription interference. The late SV40 polyA terminates the transcription of the gene of interest while the human β-globin polyA terminates the transcription of the selectable marker.

pSELECT-GFP-LC3 is a mammalian expression vector containing the human LC3B gene fused at its 5' end to the green fluorescent protein (GFP) gene. This plasmid is selectable in bacteria and mammalian cells using Zeocin™. Expression of the GFP-LC3 fusion gene allows to visualize autophagosome formation in real time in live cells. During autophagosome formation, GFP-LC3 is processed and recruited to the autophagosome membrane, where it can be imaged as cytoplasmic puncta by high resolution fluorescence microscopy. The percentage of GFP-LC3 positive cells can be determined and is indicative of autophagosome formation.

The same plasmid is available with the GFP gene alone as a control. This control plasmid is called pSELECT-NGFP-zeo (cat. code: psetz-ngfp). This plasmid is selectable in bacteria and mammalian cells with Zeocin™.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat. The EF-1α promoter exhibits a strong activity and yields long lasting expression of a transgene *in vivo*. The R-U5' has been coupled to the EF-1α core promoter to enhance stability of RNA.

- **GFP::LC3B** fusion gene was generated by fusing a GFP variant to the 5' end of the human LC3B gene. A synthetic intron was added between both moieties to increase the activity of GFP. This hybrid protein absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm).
- **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA.
- **ori:** a minimal *E. coli* origin of replication to limit vector size, but with the same activity as the longer Ori.

Second expression cassette

- **CMV enh/prom:** The human cytomegalovirus immediate-early gene 1 promoter/enhancer was originally isolated from the Towne strain and was found to be stronger than any other viral promoters.
- **EM7** is a bacterial promoter that enables the constitutive expression of the antibiotic resistance gene in *E. coli*.
- **Zeo:** Resistance to Zeocin™ is conferred by the *Sh ble* gene from *Streptoalloteichus hindustanus*. The *Sh ble* gene is driven by the CMV enhancer/promoter in tandem with the bacterial EM7 promoter allowing selection in both mammalian cells and *E. coli*.
- **βGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription¹.

METHODS

Plasmid resuspension

Quickly spin the tube containing the lyophilized plasmid to pellet the DNA. To obtain a plasmid solution at 1 µg/µl, resuspend the DNA in 20 µl of sterile H₂O. Store resuspended plasmid at -20 °C.

Plasmid amplification and cloning

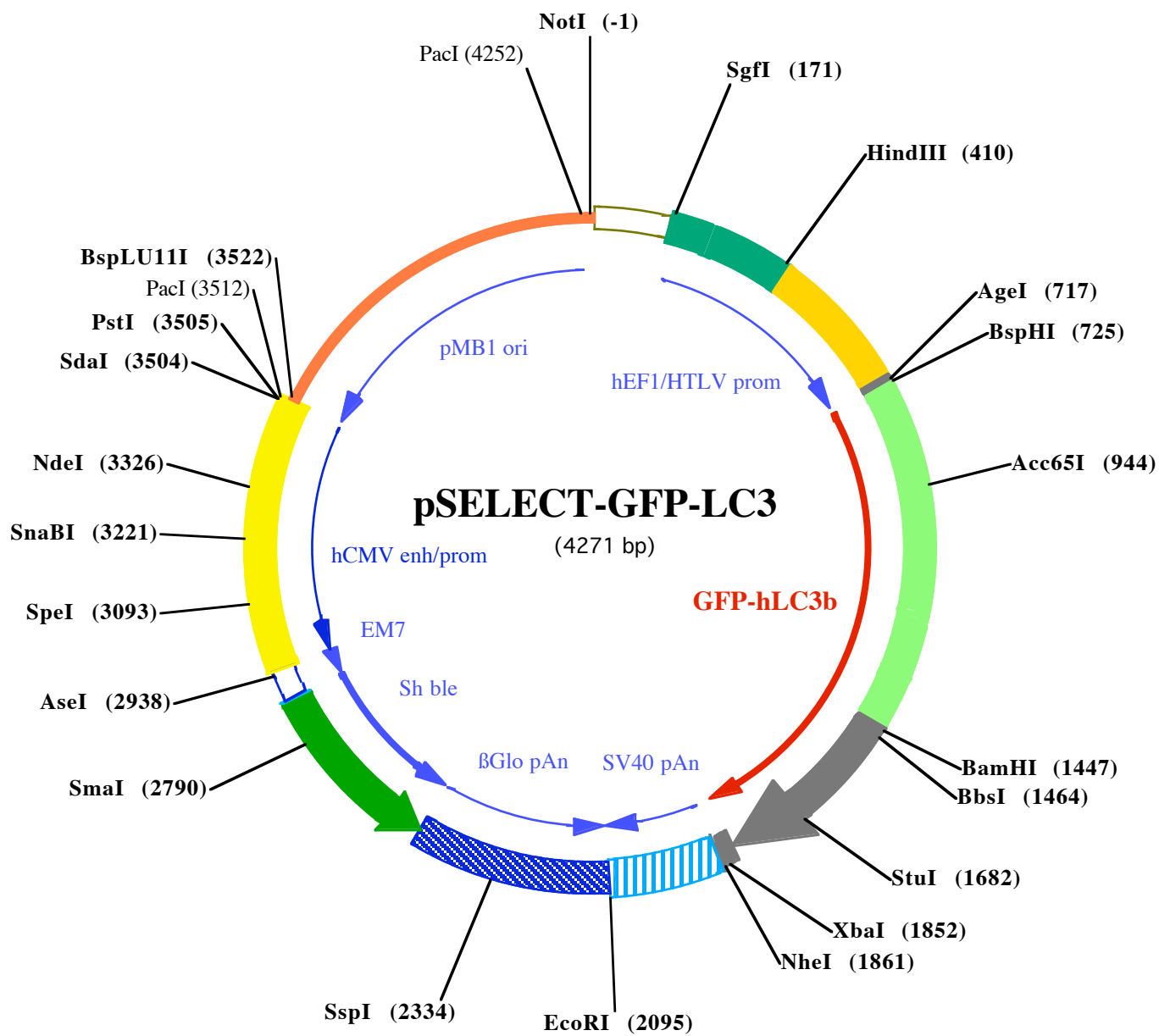
Plasmid amplification and cloning can be performed in *E. coli* GT116 or in other commonly used laboratory *E. coli* strains, such as DH5α.

Zeocin™ usage

This antibiotic can be used for *E. coli* at 25 µg/ml in liquid or solid media and at 50-200 µg/ml to select Zeocin™-resistant mammalian cells.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
E-mail: info@invivogen.com



NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG

SgfI (171)

149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCGAGCGCACATCGCCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAAGT

297 GATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGTCGCCGTGAA

HindIII (410)

371 CGTTCCTTTTTCGCAACGGGTTTTCGCGCCAGAACACAGCTGAAGCTTCGAGGGCTCGCATCTCTCCTTCACGCG

445 CCGCGCCCTACCTGAGGCCGCCATCCACGCCGGTTGAGTCGCGTTCTGCCGCTCCCGCTGTGGTGCCTCC

519 TGAAGTGCCTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCTTTGTCCGGCGCTCCCTTGGAG

593 CCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTTGTTTT

BspHI (725)

AgeI (717)

667 CTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGTCATCATGAGCAAGGGAG

1 MetSer LysGlyG

741 AAGAAGTCTTTACTGGTGTGTCCCAATTCTGGTTGAGCTGGATGGTGTGATGTGAATGGCCACAAATCTCTGTG

5 IuGIuLeuPheThrGlyValValProIleLeuValGluLeuAspGlyAspValAsnGlyHisLysPheSerVal

815 TCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAGCTGACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCC

30 SerGlyGluGlyGlyAspAlaThrTyrGlyLysLeuThrLeuLysPheIleCysThrThrGlyLysLeuP

Acc65I (944)

889 AGTGCCTTGGCCAACTCTGGTGACCACCCTGACTTATGGTGTTCATGTTTCAGCAGGTACCCTGACCACATGA

54 oValProTrpProThrLeuValThrThrLeuThrTyrGlyValGlnCysPheSerArgTyrProAspHisMetL

963 AGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGAT

79 ysGlnHisAspPhePheLysSerAlaMetProGluGlyTyrValGlnGluArgThrIlePhePheLysAspAsp

1037 GGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATACTGGTTAACAGAATTGAGCTGAAAGGCAT

104 GlyAsnTyrLysThrArgAlaGluValLysPheGluGlyAspThrLeuValAsnArgIleGluLeuLysGlyIle

1111 TGATTTTAAGGAAGATGGAACATTCTGGGTCAACAGCTGGAGTACAACCTATAATTCTCACAATGTTTACATTA

128 eAspPheLysGluAspGlyAsnIleLeuGlyHisLysLeuGluTyrAsnTyrAsnSerHisAsnValTyrIleM

1185 TGGCAGATAAGCAGAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAA

153 eAlaAspLysGlnArgAsnGlyIleLysAlaAsnPheLysIleArgHisAsnIleGluAspGlySerValGln

1259 CTGGCAGACCATTACCAGCAGAACCCTATTGGTGTGAGTGGCCAGTTCCTCCTCCAGATAATCACTATCTCAG

178 LeuAlaAspHisTyrGlnGlnAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSe

1333 CACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCCTGGAGTTTGTGACAGCAG

202 rThrGlnSerAlaLeuSerLysAspProAsnGluLysArgAspHisMetValLeuLeuGluPheValThrAlaA

BamHI (1447)

BbsI (1464)

1407 CAGGAATTACTCTGGGAATGGATGAGCTGTACAAGGGAGGTGGATCCATGCCGTGCGAGAAGACCTTCAAGCAG

227 laGlyIleThrLeuGlyMetAspGluLeuTyrLysGlyGlyGlySerMetProSerGluLysThrPheLysGln

1481 CGCCGCACCTTGAACAAAGAGTAGAAGATGTCCGACTTATTCGAGAGCAGCATCCAACCAAAATCCCGGTGAT

252 ArgArgThrPheGluGlnArgValGluAspValArgLeuIleArgGluGlnHisProThrLysIleProValIle

1555 AATAGAACGATACAAGGGTGAGAAGCAGCTTCTGTTCTGGATAAAACAAAGTTCCTTGTACCTGACCATGTCA

276 eIleGluArgTyrLysGlyGlyLysGlnLeuProValLeuAspLysThrLysPheLeuValProAspHisValA

StuI (1682)

1629 ACATGAGTGAGCTCATCAAGATAATTAGAAGGCGCTTACAGCTCAATGCTAATCAGGCCTTCTTCTGTTGGTG

301 snMetSerGluLeuIleLysIleIleArgArgArgLeuGlnLeuAsnAlaAsnGlnAlaPhePheLeuLeuVal

1703 AACGGACACAGCATGGTCAGCGTCTCCACACCAATCTCAGAGGTGTATGAGAGTGAGAAAGATGAAGATGGATT

326 AsnGlyHisSerMetValSerValSerThrProIleSerGluValTyrGluSerGluLysAspGluAspGlyPh

1777 CCTGTACATGGTCTATGCCTCCAGGAGACGTTCCGGATGAAATTGTGAGTGTAAAACAGAAAAAATGCAGCT

350 eLeuTyrMetValTyrAlaSerGlnGluThrPheGlyMetLysLeuSerVal ●●●

NheI (1861)

XbaI (1852)

1851 CTTCTAGAATTGCTAGCTGGCCAGACATGATAAGATACATTGATGAGTTTTGGACAAACCACAACCTAGAATGCAG

1925 TGAAAAAATGCTTTATTTGTGAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACA

1999 AGTTAACAAACAACAATTGCATTCAATTTATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGT

EcoRI (2095)

2073 AAAACCTCTACAAATGTGGTATGGAATTCTAAAATACAGCATAGCAAACCTTTAACCTCCAAATCAAGCCTCTA

2147 CTTGAATCCTTTTCTGAGGGATGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGA

2221 GCCTCACCTTCTTTTCATGGAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTCTCTTCATTTCTTTAT

SspI (2334)

2295 GTTTTAAATGCACTGACCTCCCACATTCCCTTTTTAGTAAAATATTAGAAAATAATTTAAATACATCATTGCAA

2369 TGAAAATAAATGTTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTT

2443 GGACTTAGGGAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCC

125↓ ●●●AspG

2517 TGCTCCTCTGCCACAAAGTGCACGCAGTTGCCGGCCGGTTCGCGCAGGGCGAACTCCCGCCCCACGGCTGCTC

122↓ | nGl uGl uAl aVal PheHi sVal CysAsnGl yAl aP roAspArgLeuAl aPheGl uArgGl yTrpP roGl nGl u

2591 GCCGATCTCGGTTCATGGCCGGCCCGAGGCGTCCCGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACA

98↓ | Gl yI l eGl uThr MetAl aP roGl ySer Al aAspArgPheAsnThr Ser Val Val Gl uSer TrpGl uAl aTyrLe

2665 GCTCGTCCAGGCCGCGCACCCACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTTGACCGCGCTGATG

73↓ | uGl uAspLeuGl yArgVal TrpVal TrpAl aLeuThrAsnAspP roVal Val Gl nAspGl nVal Al aSer l l eP

SmaI (2790)

2739 AACAGGGTCACGTCGTCCCGGACCACACCGGCGAAGTCGTCTCCACGAAGTCCCGGAGAACCCGAGCCGGTC

48↓ | heLeuThr Val AspAspArgVal Val Gl yAl aPheAspAspGl uVal PheAspArgSer PheGl yLeuArgAsp

2813 GGTCCAGAACTCGACCGCTCCGGCGACGTGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGGCCATGA

24↓ | Thr TrpPheGl uVal Al aGl yAl aVal AspArgAl aThr LeuVal P roVal Al aSer Thr LeuLysAl aMet

AseI (2938)

2887 TGGCCCTCCTATAGTGAGTCGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGG

2961 ATGGCGTCTCCAGCTTATCTGACGGTTCACATAAACGAGCTCTGCTTATATAGACCTCCCACCGTACACGCCTA

SpeI (3093)

3034 CCGCCCATTTGCGTCAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAAACA

3107 AACTCCCATTGACGTCATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATG

SnaBI (3221)

3181 TACTGCCAAAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCA

NdeI (3300)

3255 TAAGGTCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCATAGGGGGCGTACTTGGCA

3329 TATGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCATGGAAAGTC

3403 CCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCATGGGCGGGGTGCTTGGGCGGTCAGCCAGG

PacI (3512)

PstI (3505)

SdaI (3504)

BspLU11I (3522)

3477 CGGGCCATTTACCGTAAGTTATGTAACGCCTG CAG G TT AA TT AAGAACATGTGAGCAAAGGCCAGCAAAA

3548 GGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAA

3622 ATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTGGAAGCTCC

3696 CTCGTGCCTCTCCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGAAGCGTGGC

3770 GCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACG

3844 AACCCCCGTTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGAC

3918 TTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTT

3992 GAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCT

4066 TCGGAAAAGAGTTGGTAGCTCTTGATCCGGCAAACAAACCACCGCTGGTAGCGGTGGTTTTTTGTTTGCAAG

4140 CAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGGTCTGACGCTCAGTG

PacI (4252)

4214 GAACGAAAACACGTTAAGGGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A