

pSELECT-NGFP-zeo

Plasmid for the expression of GFP-N-terminal tagged proteins

Catalog code: psetz-ngfp

For research use only

Version 20K30-MM

PRODUCT INFORMATION

Content:

- 20 µg of pSELECT-NGFP-zeo plasmid provided as lyophilized DNA
- 1 ml of Zeocin™ (100 mg/ml)

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 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-Tag is a new family of expression plasmids designed to generate tagged proteins in order to facilitate their detection and/or purification. pSELECT-Tag features three well-known tags: the green fluorescent protein (GFP) gene, the human influenza hemagglutinin (HA) epitope and the polyhistidine (His) tag. The GFP gene encodes a green fluorescent protein that absorbs blue light (major peak at 480 nm) and emits green light (major peak at 505 nm). pSELECT-Tag plasmids allow to add the tag either at the N or C terminus of the protein of interest. N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS). pSELECT-NGFP-zeo can also be used as a control plasmid for pSELECT-GFP-LC3.

PLASMID FEATURES

First expression cassette

• **hEF1-HTLV prom** is a composite promoter comprising the Elongation Factor-1α (EF-1α) core promoter¹ and the R segment and part of the U5 sequence (R-U5') of the Human T-Cell Leukemia Virus (HTLV) Type 1 Long Terminal Repeat². 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.

• **MCS:** The multiple cloning site contains the following restriction sites: 5' - BamH I, Eco47III, Nco I, Nhe I - 3'

Each restriction site is compatible with many other enzymes.

• **SV40 pAn:** the Simian Virus 40 late polyadenylation signal enables efficient cleavage and polyadenylation reactions resulting in high levels of steady-state mRNA³.

• **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*.

• **Sh ble gene** confers zeocin resistance. 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*.

• **BGlo pAn:** The human beta-globin 3'UTR and polyadenylation sequence allows efficient arrest of the transgene transcription⁴.

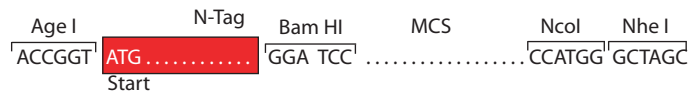
CLONING STRATEGY

For expression of a tagged protein, it is important to ensure to clone your gene-of-interest into the correct reading frame. In general it is recommended to use Bam HI/Nhe I restriction site combination for cloning into plasmids with the N-Tag. For the plasmids with N-Tag, check whether your gene of interest and the start codon of the N-Tag are in the correct reading frame.

Note: The Bam HI restriction site is compatible with Bgl II.



If it is not possible to use the Nhe I restriction site, it is possible to use another restriction site such as Nco I.



Alternatively, blunt end cloning can be achieved using the Eco 47 III site, which is downstream of the Bam HI site. Blunt end PCR fragments can be obtained using T4 DNA polymerase or Klenow.



TECHNICAL SUPPORT

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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 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.

References:

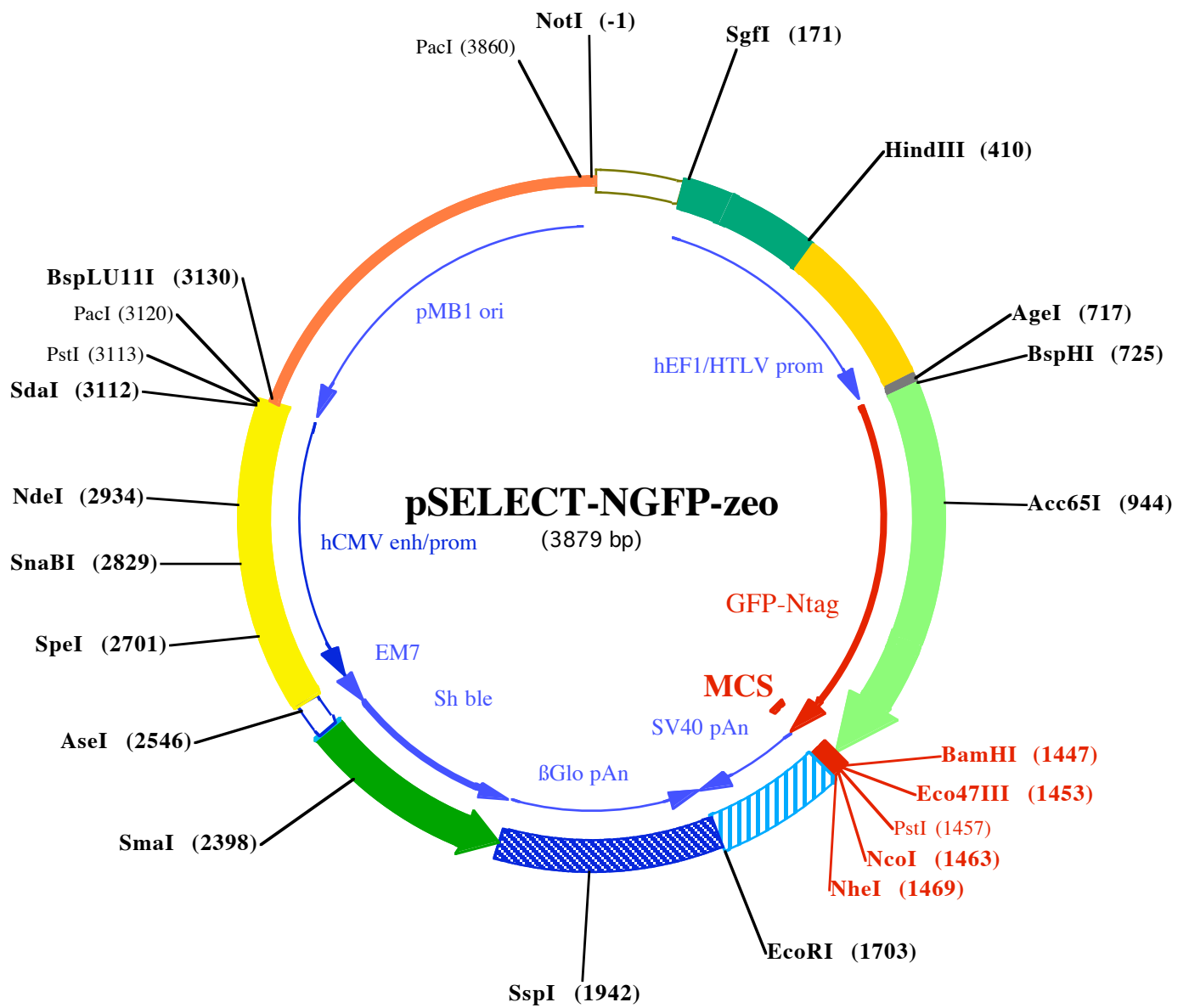
1. Kim, D.W. *et al.* (1990). *Gene* 2: 217-223.
2. Takebe, Y. *et al.* (1988). *Mol. Cell Biol.* 1: 466-472.
3. Carswell, S., and Alwine, J.C. (1989). *Mol. Cell Biol.* 10: 4248-4258.
4. Yu J & Russell JE. (2001). *Mol Cell Biol*, 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
pSELECT-NGFP-blasti	psetb-ngfp
pSELECT-CGFP-blasti	psetb-cgfp
pSELECT-CGFP-zeo	psetz-cgfp
pSELECT-NHA-blasti	psetb-nha
pSELECT-CHA-blasti	psetb-cha
pSELECT-NHA-zeo	psetz-nha
pSELECT-CHA-zeo	psetz-cha
pSELECT-CHis-blasti	psetb-chis
pSELECT-NHis-zeo	psetz-nhis
pSELECT-CHis-zeo	psetz-chis

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InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3622-3480
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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACATAC
75 GCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAAATAGGCTGTCCCCAGTGCAAGTGCAGGTGCCAG

SgfI (171)

149 AACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCC

223 CCGAGAAGTTGGGGGAGGGGTGCGCAATTGAACGGGTGCCTAGAGAAGGTGGCGGGGTAAACTGGGAAAG

296 TGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCCTATATAAGTGCAGTAGTCGCCGTGA

HindIII (410)

370 ACGTTCTTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTCGCATCTCTCCTTCACGC

444 GCCCCGCCCTACCTGAGGCCGCCATCCACGCCGTTGAGTCGCGTTCTGCCGCCCTCCCGCTGTGGTGCCTC

518 CTGAACTGCGTCCGCCGTCTAGGTAAGTTTAAAGCTCAGGTCGAGACCGGGCCTTTGTCCGGCGCTCCCTTGA

592 GCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCTGACCCTGCTTGTCAACTCTACGTCTTTGTTTTCGTTT

BspHI (725)

AgeI (717)

666 TCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACCTGAGATCACCGGCATCATGAGCAAGGGA

1 MetSer LysGly

740 GAAGAACTCTTTACTGGTGTGTCCCAATTCTGGTTGAGCTGGATGGTGTGAATGGCCACAAATCTCTGT

5 Gl uGl uLeuPheThr Gl yVal Val P roI l eLeuVal Gl uLeuAspGl yAspVal AsnGl yHi sLysPheSer Va

814 GTCTGGTGAAGGTGAAGGAGATGCAACTTATGGAAAGCTGACTCTGAAGTTTATTTGTACAACAGGAAAGCTGC

29 I Ser Gl yGl uGl yGl uGl yAspAl aThr TyrGl yLysLeuThr LeuLysPheI l eCysThr Thr Gl yLysLeuP

Acc65I (944)

888 CAGTGCCTTGCCAACTCTGGTGACCACCCTGACTTATGGTGTTCATGTTTCAGCAGGTACCCTGACCACATG

54 r oVal P roTrpP roThr LeuVal Thr Thr LeuThr TyrGl yVal Gl nCysPheSer ArgTyrP roAspHi sMet

962 AAGCAGCATGACTTCTTTAAATCTGCAATGCCAGAAGGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGA

79 LysGl nHi sAspPhePheLysSer Al aMetP roGl uGl yTyrVal Gl nGl uArgThr I l ePhePheLysAspAs

1036 TGGAAATTATAAGACAAGGGCAGAAGTGAAGTTTGAAGGTGATCACTGGTTAACAGAATTGAGCTGAAAAGGCA

103 pGl yAsnTyrLysThr ArgAl aGl uVal LysPheGl uGl yAspThr LeuVal AsnArgI l eGl uLeuLysGl yI

1110 TTGATTTTAAGGAAGATGGAACATTCTGGGTCAAGCTGGAGTACAACATAATTCTCACAATGTTTACATT

128 I eAspPheLysGl uAspGl yAsnI l eLeuGl yHi sLysLeuGl uTyrAsnTyrAsnSer Hi sAsnVal TyrI l e

1184 ATGGCAGATAAGCAGAGGAATGGAATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCA

153 MetAl aAspLysGl nArgAsnGl yI l eLysAl aAsnPheLysI l eArgHi sAsnI l eGl uAspGl ySer Val Gl

1258 ACTGGCAGACCATTACCAGCAGAACACCCCTATTGGTGTGATGGCCAGTTCTCCTCCAGATAATCACTATCTCA

177 nLeuAl aAspHi sTyrGl nGl nAsnThr P roI l eGl yAspGl yProVal l eLeuLeuP roAspAsnHi sTyrLeuS

1332 GCACTCAATCTGCTCTGTCCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCCTGGAGTTTGTGACAGCA

202 er Thr Gl nSer Al aLeuSer LysAspP roAsnGl uLysArgAspHi sMetVal l eLeuLeuGl uPheVal Thr Al a

PstI (1457)

Eco47III (1453)

NheI (1469)

BamHI (1447)

NcoI (1463)

1406 GCAGGAATTACTCTGGGAATGGATGAGCTGTACAAGGGAGGTGGATCCAGCGCTGCAGCCATGGGCTAGCTGGC

227 Al aGl yI l eThr LeuGl yMetAspGl uLeuTyrLysGl yGl yGl ySer Ser Al aAl aAl aMetGl y••• -

1480 CAGACATGATAAGATACATTGATGAGTTTGACAAAACCACAACACTAGAATGCAGTAAAAAAATGCTTTATTTGT

1554 GAAATTTGTGATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAAACAAGTTAACAACAACAATTGCAT

1628 TCATTTTATGTTTCAGGTTTCAGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTA

EcoRI (1703)

1702 TGGAATTCTAAAATACAGCATAGCAAACTTTAACCTCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGA

1776 TGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATGGAG

1850 TTTAAGATATAGTGTATTTTCCAAGGTTTGAAGTACTGCTCTTCATTTCTTTATGTTTTAAATGCACTGACCTCC

SspI (1942)

1924 CACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATGTTTTTTATT
 1998 AGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGGGAACAAAGGAAC
 2072 CTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTATCCTCAGTCCTGCTCCTCTGCCACAAAGTGC
 2146 ACGCAGTTGCCGGCCGGTGCAGCAGGGCGAACTCCCGCCCCACGGCTGCTCGCCGATCTCGGTCATGGCCGG
 115↓ a l CysAsnG l yAl aProAspArgLeuAl aPheG l uArgG l yTrpP roG l nG l uG l y l eG l uThr M e tAl aP ro
 2220 CCCGGAGGCGTCCCGGAAGTTCGTGGACACGACCTCCGACCACTCGGCGTACAGCTCGTCCAGGCCGCGCACCC
 91↓ G l ySer Al aAspArgPheAsnThr Ser Val Val G l uSer TrpG l uAl aTyrLeuG l uAspLeuG l yA rgVal Tr
 2294 ACACCCAGGCCAGGGTGTGTCCGGCACCACTGGTCTGGACCGCGCTGATGAACAGGGTCACGTCGTCCCGG
 66↓ pVal TrpAl aLeuThrAsnAspProVal Val G l nAspG l nVal Al aSer l l ePheLeuThr Val AspAspArgV

SmaI (2398)

2368 ACCACACCGGCGAAGTCGTCCTCCACGAAGTCCCGGGAGAACCCGAGCCGGTCCGGTCCAGAACTCGACCGCTCC
 41↓ a l Val G l yAl aPheAspAspG l uVal PheAspArgSer PheG l yLeuArgAspThr TrpPheG l uVal Al aG l y
 2442 GCGGACGTCGCGCGCGGTGAGCACCGGAACGGCACTGGTCAACTTGCCATGATGGCCCTCTATAGTGAGTGC
 17↓ Al aVal AspArgAl aThr LeuVal ProVal Al aSer Thr LeuLysAl aM e t ←

AseI (2546)

2516 TATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTATCT
 2589 GACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTTGCGTCAATGGG

SpeI (2701)

2663 GCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAAACAAACTCCCATTGACGTCAATGG
 2736 GGTGGAGACTTGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCAAAACCGCATCAT

SnaBI (2829)

2809 CATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAAGGTCATGTACTGGGCAT

NdeI (2934)

2883 AATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATATGATACACTTGATGTACT
 2957 GCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGTCCCTATTGGCGTACTATGGG
 3031 AACATACGTCATTATTGACGTCAATGGGCGGGGGTCTGTTGGGCGGTGAGCCAGGCGGGCCATTTACCGTAAAGTT

PstI (3113) PacI (3120)

SdaI (3112)

BspLU11I (3130)

3105 ATGTAACGCCTG CAG G TT AA TT AAGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGG
 3176 CCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGG
 3250 TGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTTCCCCTGGAAGCTCCCTCGTGCGCTCTCCTGTTCC
 3324 GACCCTGCCGTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGAAGCGTGCGCTTTTCTCATAGCTCACGCT
 3398 GTAGGTATCTCAGTTCGGTGTAGGTGCTTCGCTCCAAGCTGGGCTGTGTGCACGAACCCCCGTTTCAGCCCGAC
 3472 CGCTGCGCCTTATCCGGTAACTATCGTCTTGAGTCCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGC
 3546 CACTGGTAACAGGATTAGCAGAGCGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACG
 3620 GCTACACTAGAAGAACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGA AAAAGAGTTGGTAGC
 3694 TCTTGATCCGGCAAACAACACCCTGCTGCTGAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAA
 3768 AAAAGGATCTCAAGAAGATCCTTTGATCTTTTCTACGGGTCTGACGCTCAGTGGAACGAAAACCTCACGTTAAG

PacI (3860)

3842 GGATTTTGGTCATGGCTAGTTAATTAACATTTAAATC A