

pSELECT-NGFP-blasti

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

Catalog code: psetb-ngfp

<https://www.invivogen.com/pselect-gfp-tag>

For research use only

Version 20111-MM

PRODUCT INFORMATION

Contents

- 20 µg of pSELECT-NGFP-blasti plasmid provided as lyophilized DNA
- 2 x 1 ml blasticidin at 10 mg/ml

Storage and stability

- Product is shipped at room temperature.
- Upon receipt, store lyophilized DNA at -20°C.
- Resuspended DNA should be stored at -20°C.
- Store blasticidin at 4°C or -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. The N-terminal tag: the tag encompasses the Start codon and is followed by a multiple cloning site (MCS).

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' - Bam HI, Eco 47III, 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*.

- **Bsr:** Resistance to Blasticidin S is conferred by the *bsr* gene from *Bacillus cereus*. The *bsr* 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 transcription⁴.

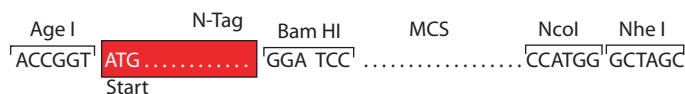
CLONING STRATEGY

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 or other commonly used laboratory *E. coli* strains, such as DH5α.

Blasticidin usage

Blasticidin should be used at 25-100 µg/ml in bacteria and 1-30 µg/ml in mammalian cells. Blasticidin is supplied at 10 mg/ml in HEPES buffer.

References:

1. Kim, D.W. et al., 1990. Use of the human elongation factor 1 alpha promoter as a versatile and efficient expression system. *Gene* 2: 217-223. 2. Takebe, Y. et al., 1988. SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. *Mol. Cell Biol.* 1: 466-472. 3. Carswell, S., & Alwine, J.C., 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: effects of upstream sequences. *Mol. Cell Biol.* 10: 4248-4258. 4. Yu J & Russell JE., 2001. Structural and functional analysis of an mRNP complex that mediates the high stability of human beta-globin mRNA. *Mol Cell Biol.* 21(17):5879-88.

RELATED PRODUCTS

Product	Catalog Code
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

TECHNICAL SUPPORT

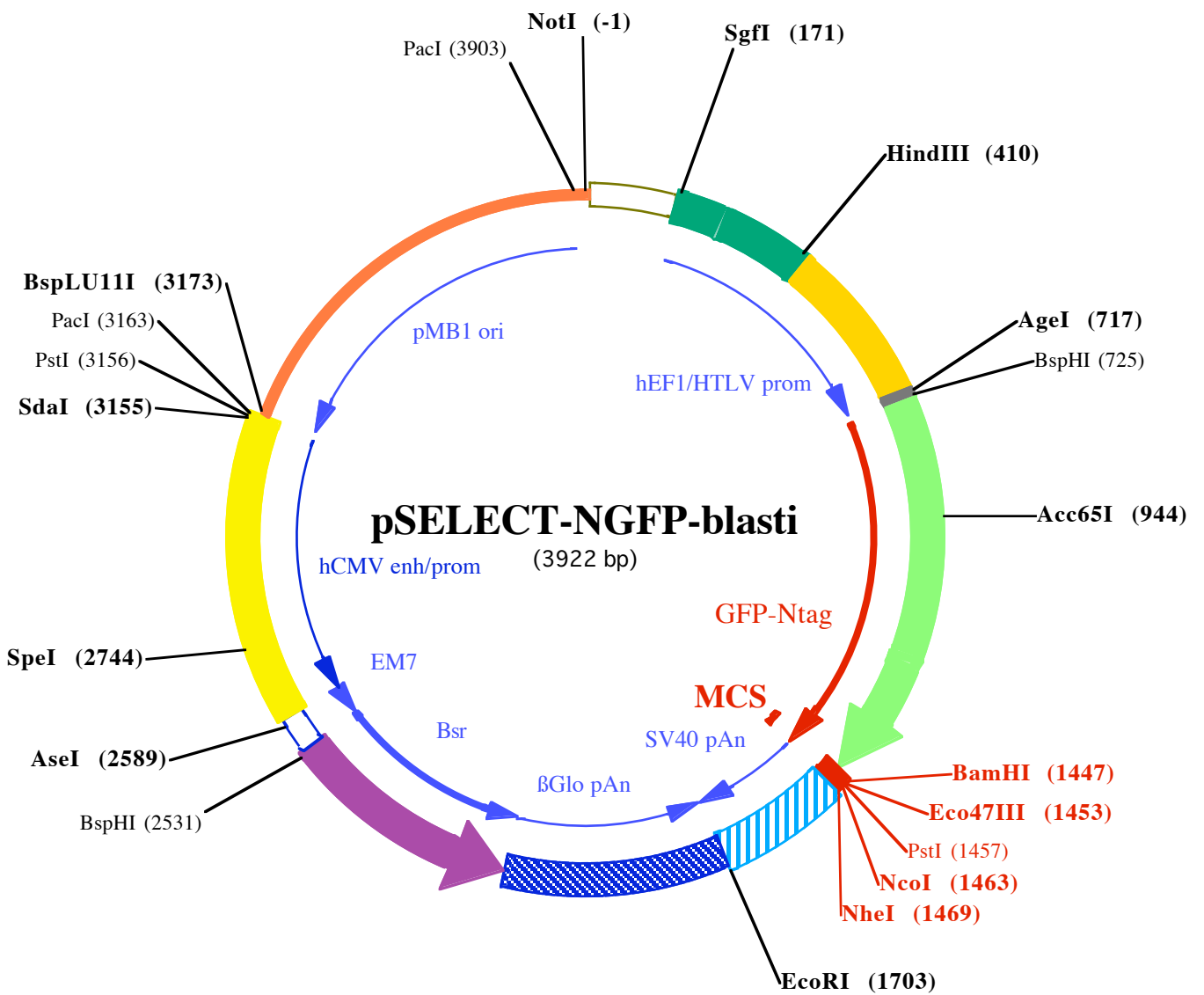
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NotI (-1)

1 GCGGCCGCAATAAAATATCTTTATTTTCATTACATCTGTGTGTTGGTTTTTTGTGTGAATCGTAACTAACA
72 TACGCTCTCCATCAAAACAAAACGAAACAAAACAACTAGCAAATAGGCTGTCCCCAGTGCAAGTGCAGG

SgfI (171)

143 TGCCAGAACATTTCTCTATCGAAGGATCTGCGATCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGC

214 CCACAGTCCCCGAGAAGTTGGGGGGAGGGGTGCGCAATTGAACGGTGCCTAGAGAAGGTGGCGCGGGGTA

285 AACTGGGAAAGTGATGTCGTGTACTGGCTCCGCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGC

HindIII (410)

356 AGTAGTCGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGCTGAAGCTTCGAGGGGCTC

427 GCATCTCTCCTTCACGCGCCCGCCCTACCTGAGGCCGTCATCCACGCCGGTTGAGTCGCGTTCTGCCG

498 CCTCCCGCCTGTGGTGCCTCCTGAACTGCGTCCGCGTCTAGGTAAGTTTAAAGCTCAGGTGCGAGACCGG

569 CCTTTGTCCGGCGCTCCCTTGAGCCTACCTAGACTCAGCCGGCTCTCCACGCTTTGCCTGACCCTGCTTG

640 CTCAACTCTACGTCTTTGTTTCTGTTCTGCGCCGTTACAGATCCAAGCTGTGACCGGCGCCTACC

BspHI (725)

AgeI (717)

711 TGAGATCACCGGTCATCATGAGCAAGGGAGAAGAAGCTTTACTGGTGTGTCCAATTCTGGTTGAGCTG

1 MetSer LysGlyValLeuPheThrGlyValValProIleLeuValGlyLeu

782 GATGGTGTATGTAATGGCCACAAATTCTCTGTGTCTGGTGAAGGTGAAGGAGATGCAACTTATGAAAAGCT

19 AspGlyAspValAsnGlyHisLysPheSerValSerGlyGlyGlyGlyAspAlaThrTyrGlyLysLeu

853 GACTCTGAAGTTCATTTGTACAACAGGAAAGCTGCCAGTGCCTTGCCAACTCTGGTGACCACCTGACTT

42 uThrLeuLysPheIleCysThrThrGlyLysLeuProValProTropProThrLeuValThrThrLeuThrT

Acc65I (944)

924 ATGGTGTTCATGTTTCAGCAGGTACCCTGACCACATGAAGCAGCATGACTTCTTTAAATCTGCAATGCCA

66 yrGlyValGlnCysPheSerArgTyrProAspHisMetLysGlnHisAspPhePheLysSerAlaMetPro

995 GAAGTTATGTTTCAGGAGAGGACAATCTTCTTTAAGGATGATGGAAATTATAAGACAAGGGCAGAAAGTAA

90 GlyGlyTyrValGlnGluArgThrIlePhePheLysAspAspGlyAsnTyrLysThrArgAlaGluValLys

1066 GTTTGAAGGTGATACACTGGTTAACAGAATTGAGCTGAAAGGCATTGATTTTAAGGAAGATGGAAACATTC

113 sPheGlyGlyAspThrLeuValAsnArgIleGluLeuLysGlyIleAspPheLysGlyAspGlyAsnIleL

1137 TGGGTCACAAGCTGGAGTACAACATAATTCTCACAATGTTTACATTATGGCAGATAAGCAGAGGAATGGA

137 euGlyHisLysLeuGlyTyrAsnTyrAsnSerHisAsnValTyrIleMetAlaAspLysGlnArgAsnGly

1208 ATTAAGGCTAATTTCAAGATTAGACACAACATTGAGGATGGATCTGTCCAACCTGGCAGACCATTACCAGCA

161 IleLysAlaAsnPheLysIleArgHisAsnIleGluAspGlySerValGlnLeuAlaAspHisTyrGlnGly

1279 GAACACCCCTATTGGTGTATGGCCAGTCTCCTCCAGATAATCACTATCTCAGCACTCAATCTGCTCTGT

184 nAsnThrProIleGlyAspGlyProValLeuLeuProAspAsnHisTyrLeuSerThrGlnSerAlaLeuS

1350 CCAAAGACCCTAATGAGAAAAGAGACCACATGGTCTCCTGGAGTTTGTGACAGCAGCAGGAATTAAGTCTG

208 erLysAspProAsnGlyLysArgAspHisMetValLeuLeuGlyPheValThrAlaAlaGlyIleThrLeu

PstI (1457)

Eco47III (1453) NheI (1469)

BamHI (1447) NcoI (1463)

1421 GGAATGGATGAGCTGTACAAGGGAGGTGGATCCAGCGCTGCAGCCATGGGCTAGCTGGCCAGACATGATAA

232 GlyMetAspGlyLeuTyrLysGlyGlyGlySerSerAlaAlaAlaMetGly•••

1492 GATACATTGATGAGTTTGACAAACCACAACCTAGAATGCAGTGAAAAAATGCTTTATTTGTGAAATTTGT

1563 GATGCTATTGCTTTATTTGTAACCATTATAAGCTGCAATAACAAGTTAACAACAACAATTGCATTCATT

EcoRI (170)

1634 TATGTTTCAGGTTTCAGGGGGAGGTGTGGGAGGTTTTTTAAAGCAAGTAAAACCTCTACAAATGTGGTATGG

1705 AATTCTAAAATACAGCATAGCAAACCTTTAACCTCCAAATCAAGCCTCTACTTGAATCCTTTTCTGAGGGA

1776 TGAATAAGGCATAGGCATCAGGGGCTGTTGCCAATGTGCATTAGCTGTTTGCAGCCTCACCTTCTTTCATG

1847 GAGTTTAAGATATAGTGTATTTTCCCAAGGTTTGAAGTGTGACTGCTCTTCATTTCTTTATGTTTTAAATGCACTG

1918 ACCTCCCACATTCCCTTTTTAGTAAAATATTCAGAAATAATTTAAATACATCATTGCAATGAAAATAAATG
 1989 TTTTTTATTAGGCAGAATCCAGATGCTCAAGGCCCTTCATAATATCCCCAGTTTAGTAGTTGGACTTAGG
 2060 GAACAAAGGAACCTTTAATAGAAATTGGACAGCAAGAAAGCGAGCTTCTAGCTTTAGTTCTGGTGTACTT
 2131 GAGGGGGATGAGTTCCTCAATGGTGGTTTTGACCAGCTTGCCATTCATCTCAATGAGCACAAAGCAGTCAG
 135↓ LeuProI leLeuGl uGl uI leThr Thr LysVal LeuLysGl yAsnMetGl uI leLeuVal PheCysAspP r
 2202 GAGCATAGTCAGAGATGAGCTCTCTGCACATGCCACAGGGGCTGACCACCCTGATGGATCTGTCCACCTCA
 111↓ oAl aTyrAspSer I leLeuGl uArgCysMetGl yCysProSer Val Val A rgl I leSer ArgAspVal Gl uA
 2273 TCAGAGTAGGGGTGCTGACAGCCACAATGGTGTCAAAGTCTTCTGCCGTTGCTCACAGCAGACCCAAT
 87↓ spSer TyrProHi sArgValAl aVal I leThrAspPheAspLysGl nGl yAsnSer ValAl aSer Gl yI le
 2344 GGCAATGGCTTCAGCACAGACAGTGACCCTGCCAATGTAGGCCTCAATGTGGACAGCAGAGATGATCTCCC
 64↓ Al aI leAl aGl uAl aCysVal Thr Val ArgGl yI leTyrAl aGl uI leHi sValAl aSer I leI leGl uGl
 2415 CAGTCTTGGTCTGATGGCCGCCCCGACATGGTGTCTTGTCTCATAGAGCATGGTGTCTTCTCAGTG
 40↓ yThr LysThr ArgI leAl aAl aGl yVal Hi sHi sLysAsnAspGl uTyrLeuMetThr I leLysGl uThr A
 2486 GCGACCTCCACCAGCTCCAGATCCTGCTGAGAGATGTTGAAGTCTTCATGATGGCCCTCCTATAGTGAGT
 BspHI (2531)
 16↓ I aVal Gl uVal LeuGl uLeuAspGl nGl nSer I leAsnPheThr LysMet
 AseI (2589)
 2557 CGTATTATACTATGCCGATATACTATGCCGATGATTAATTGTCAAACAGCGTGGATGGCGTCTCCAGCTT
 2628 ATCTGACGGTTCACTAAACGAGCTCTGCTTATATAGACCTCCACCGTACACGCCTACCGCCATTGCGT
 SpeI (2744)
 2699 CAATGGGGCGGAGTTGTTACGACATTTTGGAAAGTCCCGTTGATTTACTAGTCAAAAACAAACTCCCATTG
 2769 ACGTCAATGGGGTGGAGACTTGGAAATCCCCGTGAGTCAAACCGCTATCCACGCCATTGATGTACTGCCA
 2840 AAACCGCATCATCATGGTAATAGCGATGACTAATACGTAGATGTACTGCCAAGTAGGAAAGTCCCATAAGG
 2911 TCATGTACTGGGCATAATGCCAGGCGGGCCATTTACCGTCATTGACGTCAATAGGGGGCGTACTTGGCATA
 2982 TGATACACTTGATGTACTGCCAAGTGGGCAGTTTACCGTAAATACTCCACCCATTGACGTCAATGGAAAGT
 3053 CCCTATTGGCGTTACTATGGGAACATACGTCATTATTGACGTCAATGGGCGGGGGTCTGTTGGGCGGTCAGC
 PstI (3156) PacI (3163)
 SdaI (3155) BspLU11I (3173)
 3124 CAGGCGGGCCATTTACCGTAAGTTATGTAACGCCTG CAG G TT AA TT AAGAACATGTGAGCAAAAGGCCA
 3193 GCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCTGACGAGC
 3264 ATCACAAAATCGACGCTCAAGTCAGAGGTGGCGAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCC
 3335 CCTGGAAGCTCCCTCGTGCCTCTCTGTTCCGACCCTGCCGTTACCGGATACCTGTCCGCTTTTCTCCC
 3406 TTCGGGAAGCGTGGCGCTTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTTCGCTCCA
 3477 AGCTGGGCTGTGTGCACGAACCCCCGTTACGCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAG
 3548 TCCAACCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAGCGAGGTA
 3619 TGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAAGAACAGTATTTGGTA
 3690 TCTGCGCTCTGCTGAAGCCAGTTACCTTCGAAAAAGAGTTGGTAGCTCTTGATCCGGCAAAACAAACCACC

3761 GCTGGTAGCGGTGGTTTTTTTTGTTTGCAAGCAGCAGATTACGCGCAGAAAAAAGGATCTCAAGAAGATCC

3832 TTTGATCTTTTCTACGGGGTCTGACGCTCAGTGGAAACGAAAACACGTTAAGGGATTTTGGTCATGGCTA

PacI (3903)

3903 GTTAATTAACATTTAAATC A
